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THE UNIVERSITY OF ALBERTA

THE USE OF FRESHWATER ORGANISMS IN THE  
TEACHING OF SECONDARY BIOLOGY

by



KENNETH G. JACKNICKE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
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UNIVERSITY OF ALBERTA  
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Use Of Freshwater Organisms In The Teaching Of Secondary Biology," submitted by Kenneth G. Jacknicke, in partial fulfilment of the requirements for the degree of Master of Education.





## ABSTRACT

A survey of the biology teachers was conducted in the County of Lacombe during May and June, 1967, to determine the extent to which they were using local resources in the teaching of biology. The results of the survey indicated that most teachers were making very limited use of local organisms. Further, an investigation of sixty-five locations in the County of Lacombe and other areas of the province was conducted during May and June, 1967, to determine what freshwater organisms were available. A total of twenty-seven different kinds of freshwater invertebrates were found readily available in most areas.

Living specimens were collected from many of the areas investigated and attempts to culture the organisms were carried out. It was found that many of the organisms can be collected and cultured with relative ease. Successful cultures of organisms were maintained for periods ranging from several days to eleven months.

During May, June, July, and September, 1967, thirty-six mud samples were collected to determine whether or not mud can be used as a source of living materials during mid-winter months. The freezing and drying of samples, water types, container size, date of collection, and the presence of vegetation in the samples were investigated in an effort to determine their effects upon the success of specific cultures.



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A small number of mud samples were collected during February, 1968, to determine whether mud collected during mid-winter was capable of providing a source of live specimens. It was found that cultures obtained using mud collected during mid-winter produced such organisms as cladocerans, copepods, ostracods, and mayflies.

A literature review revealed a variety of methods by which live organisms can be used to aid in the teaching of biological concepts. Much of the material was found to apply to aquatic forms indigenous to Alberta.



## ACKNOWLEDGMENTS

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## CHAPTER I

### INTRODUCTION

#### I. PURPOSE OF THE STUDY

A purpose of the study was to conduct an interview in the County of Lacombe to determine to what extent teachers in that region are using local resources in the teaching of biology. Further, a survey of the County of Lacombe, and some other parts of Alberta, was conducted to determine what local organisms are available, where they are located, and how they can be readily obtained. In addition, organisms were collected to determine methods of culturing live material throughout the winter months, and to determine various ways in which local organisms can be used to facilitate instruction in biology.

Much of the study concerned the investigation of representatives of freshwater habitats. It was found that major groups of animals and plants living in this environment can be collected relatively easily and without the use of elaborate collecting equipment. Moreover, freshwater habitats are common features of the Alberta environment, and even in large populated urban areas there are often aquatic habitats to be found which will support flourishing communities of freshwater organisms.

Many biology teachers agree that the use of live materials as teaching aids is an excellent practice, yet Taylor (1965) states that



"Eighty to eighty-five percent of the teachers of high school biology are unacquainted with the most easily available and most versatile specimens for dynamic instruction in "aliveness"." Taylor does not state how this figure was obtained, but it may well be indicative of the lack of use of local resources by biology teachers. The conducted interview supported the idea that most biology teachers agree with the concept of "live-organism teaching," but it would appear that few know how to collect, maintain, and use local organisms advantageously.

## II. LIMITATIONS OF THE STUDY

Because of the large number of organisms represented in such a large and diverse area as the Province of Alberta and to facilitate handling of the information which was gathered, it was felt necessary to restrict the investigation by omitting terrestrial insects, birds, spiders, and mammals from the study. The organisms that are included in the study are members of some vertebrate and invertebrate groups. In order to prove useful to classroom teachers it seems reasonable to assume that the organisms used should be common and readily available. Therefore, organisms which are rare or difficult to collect and maintain are not included in this study.

The study interview centered in the County of Lacombe. This area was chosen because it is approximately in the center of the populated region of the province and has a wide range of environmental



conditions. Other areas of the province were examined less intensively to determine whether or not many of the organisms considered can be found province-wide.

### III. QUESTIONS RELATED TO THE STUDY

The study attempted to answer the following questions:

1. To what extent are the grade VIII and XI teachers of the County of Lacombe utilizing the local environment in the teaching of biology?
2. What reasons do teachers from the County of Lacombe give for using, or not using, local resources?
3. What common and readily available organisms are present in the County of Lacombe and several other parts of Alberta?
4. What are some of the methods by which available organisms may be collected and preserved?
5. How can collected organisms be maintained in the laboratory for extended periods of time?
6. How can local organisms be used to illustrate basic biological concepts?

The answers to questions one and two were found by conducting a personal interview with the total population of grade VIII and XI biology teachers of the County of Lacombe. Questions three and four were answered as fully as possible by extensive field investigations





which took place during May and June, 1967. Questions five and six were answered by experimentation through the fall of 1967 and winter of 1968. Culture methods and techniques for utilization of materials tried by other investigators, such as Brandwein (1966) are included.

#### IV. DEFINITION OF TERMS

A number of terms used in the study need clarification in order to avoid misinterpretation by the reader.

##### Common

Organisms which were present in over forty percent of the locations investigated will be considered plentiful enough to be termed common.

##### Frequent

Organisms which were present in ten to forty percent of the locations visited will be considered plentiful enough to be termed frequent.

##### Localized

Organisms which are considered useful but were found in less than ten percent of the areas investigated will be considered to be localized.



### Easily Accessible

Organisms are termed easily accessible if they are obtainable from locations relatively near populated centers. An attempt was made to take the majority of samples from locations within two miles of school sites. Over ninety percent of the locations investigated are within ten minutes driving time by automobile or bus to a school.

### Slough

A wet lowland area surrounded by aquatic vegetation, such as Scirpus and Typha, and some small trees. Most of the vegetation is rooted in stagnant water.

### Pond

A small quiet body of water with rooted water plants growing shore to shore.

### Flooded Excavation

An area which has been man-made, such as a gravel pit, which has become flooded. Such excavations often have relatively little vegetation growing in and around the water.

### Stream

Any natural watercourse up to fifteen feet wide.

### River

Any natural watercourse over fifteen feet wide.



### Dystrophic Water

Water which is usually dark brown in color and having a high concentration of organic matter and humus; usually very acid and has very little dissolved oxygen.

### Eutrophic Water

Water which can sustain much plant growth, has an abundant supply of basic nutrients, and is slightly alkaline or neutral.

### Oligotrophic Water

Water which is poor in dissolved nutrients, such as potassium, nitrogen, and calcium. It has little dissolved organic material, and can support only small amounts of planktonic life.

## V. RELATED LITERATURE

After investigating material available on this topic, it can be stated with reasonable certainty that there had not been a similar or related study done in the Province of Alberta previously. One thesis was found dealing with live-organism teaching. This was conducted by Bennet (1961) at the University of Florida, but deals specifically with live resources in the field of botany. The literature review, however, included a number of sourcebooks, texts, and periodicals which have proven very useful during this study.

Brandwein, Morholt, and Joseph (1966) have published A Sourcebook for the Biological Sciences which contains information on



the collecting and culturing of living organisms. Pond Life, (Engelhardt, 1964) is a British publication but many of the species mentioned are of the same genera as species found in Alberta. Freshwater Invertebrates of the United States by Pennak (1953), is a comprehensive work on the life cycles, anatomy, physiology, and identification of a large number of invertebrates common to the United States. There are available a number of invertebrate keys, such as those of Needham (1962), and Macan (1959). These keys were found useful for the identification of species.

The American Association for the Advancement of Science held a symposium and published a series of papers on Culture Methods for Invertebrate Animals (1937). Articles published in periodicals, such as The American Biology Teacher, School Science and Mathematics, and Science Teacher, were also found useful for culturing techniques and utilization of specimens.

Other sources available related to some aspects of Alberta fauna include works by Soper (1964), Farley (1932), Rowan (1963), and Salt and Wilk (1958). There are at present no available sources dealing specifically with the invertebrates of Alberta, except for a chapter in Alberta - A Natural History, (Chapter 10, Hartland-Rowe, 1967).





## CHAPTER II

### METHODS AND PROCEDURES USED IN CONDUCTING THE STUDY

An interview was conducted to determine the current utilization of local resources in the teaching of biology at the grade VIII and grade XI level in the County of Lacombe. It was decided that a personal interview of the teachers would prove superior to a questionnaire for several reasons. Firstly, there is the possibility of getting a relatively poor percentage of the questionnaires returned. Secondly, it seemed that more accurate and more detailed information might be secured in this way, particularly from teachers who were making limited use of local resources. Thirdly, a questionnaire designed to determine what organisms were being used from the many available might prove cumbersome and difficult to use. Lastly, it was felt that teachers might well respond more freely in an interview situation and thus provide the investigator with broader more meaningful information.

The interview consisted of six questions which were designed to provide the data required to arrive at rather definite generalizations. The interview sheets were numbered and coded so that a teacher's responses were anonymous. The date, school and grade level were recorded on each interview sheet. The number of classes and size of classes were also obtained in order to determine how many students were being taught by each teacher. The questions were



designed to elicit a free response from the teacher.

# 1. EXPLANATION OF INTERVIEW QUESTIONS

1. "What organisms do you collect locally and make use of with your classes in the teaching of biological concepts?"

This question was framed to determine what local resources the teacher was using. Any local organism that the teacher collected or used was noted. To qualify as a collected organism, it must have been collected at least once during the school year, from September 1966 to the time of the interview during the months of May and June, 1967.

2. "Where and how do you obtain these organisms?"

'These organisms' referred to the organisms which were listed under question one. From the answers given by the teachers it was possible to determine who collected the organisms; the teacher, the students, or the class as a whole. It was also possible to determine what equipment was used, and what locations were visited.

3. "List those organisms which you culture for study. What methods do you use?"

The question was worded to determine what organisms were cultured and the methods used for culturing. To qualify as a culture, the live organisms had to be kept living in the classroom or laboratory for a period of at least one week.



4. "How do you use these organisms which you collect locally or culture?"

Many of the organisms which are found locally can be used in many ways in the classroom to teach a wide range of biological concepts. The answers obtained from the teachers would indicate to what extent they used the organisms collected.

5. "What do you visualize as the major reasons why teachers use local resources?"

6. "What do you visualize as the major reasons why teachers may not use local resources extensively?"

Questions five and six were used to determine the individual teachers own reasons for using, or not using, local resources. The answers given by the teachers involved were compared to determine if there were any reasons which were common to the majority of the teachers. The answers obtained were also used as guidelines for further expansion into varying aspects of the study.

## II. PILOT STUDY

A pilot study of the interview was conducted with ten teachers from the Edmonton Public School Board, May, third and fourth, 1967. Six of the ten teachers were from Division IV and the remaining four teachers were currently teaching in Division III. The purpose of the





pilot study was to determine whether or not the questions on the interview sheets were appropriate to obtain the desired information. The sample was purposely biased in order to interview and obtain reactions from teachers who were known to collect and culture organisms and teachers who used live materials infrequently. On the basis of the results obtained from the pilot study, it was decided that the interview questionnaire would probably serve the desired purpose adequately. Therefore the questionnaire was used in the interviewing of teachers in the County of Lacombe, (Appendix II).

### III. METHOD OF INTERVIEW

The interview was conducted in the County of Lacombe which is situated approximately ninety miles south of Edmonton, Alberta. All teachers who were involved in teaching Biology 20 or grade eight science, which devotes approximately ten weeks to the study of animals, were included in the interview. The County of Lacombe has eleven schools situated within its boundaries. Of the eleven schools, Bentley Elementary and Lacombe Elementary were omitted because they contain only Division I and Division II students. The schools included in the study were Alix, Bentley, Blackfalds, Clive, Eckville, Lacombe High, Lacombe Junior High, Mirror and Satinwood. These nine schools served approximately 2850 students during the 1966-67 school year. Of these, approximately 455 students were enrolled in a course which offered some biology. The total number of teachers involved in





the study was eleven (Table 1).

The interviews were conducted during May and June, 1967. Each school was visited and the teachers involved were interviewed personally by the investigator and invited to answer freely to the questions on the interview sheet. The teachers were informed that the interview sheets were coded and that the information obtained would not be linked to specific teachers. An attempt was made to establish a permissive atmosphere during the interview.

#### IV. METHOD OF FIELD INVESTIGATION

The major portion of the first investigation took place during May and June, 1967. It was felt that any organisms which were collected should be available during the months when school is in session. Many aquatic environments were investigated to determine what organisms were present. There were however, a number of criteria which were considered when deciding what specific areas to investigate. Firstly, the area must be easily accessible by automobile. Secondly, as many of the samples as possible were taken from areas which were within two miles of populated centers which contained schools. Thirdly, the materials collected must be easily accessible. Only organisms which were within approximately ten feet of shore were collected.

The area investigated was bounded on its north-west corner by Peace River, on the north-east corner by Cold Lake, south-west by



TABLE I

SCHOOLS VISITED IN THE COUNTY OF LACOMBE  
TO INTERVIEW TEACHERS

SCHOOL	NO. OF PUPILS	NO. OF PUPILS TAKING BIO.20 OR GRADE 8	NO. OF TEACHERS INTERVIEWED	DATE OF INTERVIEW
Alix	350	40	1	May 16
Bentley Jr.-Sr. High	275	64	2	May 15
Blackfalds	280	25	1	May 16
Clive	234	28	1	May 16
Eckville	510	74	2	May 15
Lacombe Sr. High	520	84	1	May 15
Lacombe Jr. High	330	96	1	May 15
Mirror	275	40	1	May 16
Satinwood	85	4	1	June 25
TOTAL	2859	455	11	



Coleman, and south-east by Milk River. The date and locations examined appear in Table II and Figure 1. Organisms which were found in the aquatic samples were noted in a field book. In order to carry out investigations of culture methods, samples containing organisms were collected from approximately half of the areas investigated.

## V. EQUIPMENT USED

The equipment was kept as simple as possible in order to allow for the fact that elaborate equipment may not be available to the majority of teachers. Much of the collecting was carried out by means of a simple dip net constructed from a coat hanger and a nylon stocking. The hanger was straightened out and a loop, approximately six inches in diameter, was formed at one end. A piece of the nylon, approximately seven inches square, was then stretched tight over the loop and sewn on. Because the mesh of the nylon is so fine, many very small organisms could be caught in the net. It was found that the tight net facilitated the removal of small organisms. A looser, funnel-shaped net was utilized only if larger organisms, such as fish, were to be caught.

When obtaining a sample, the net was drawn through the water with a continuous motion. Organisms caught in the net were transferred to either a thirty-two ounce or a sixteen ounce jar containing water from which the sample was taken. In order to remove the



TABLE 11

LOCATION AND DESCRIPTION OF AREAS INVESTIGATED IN ORDER OF DATE VISITED, 1967

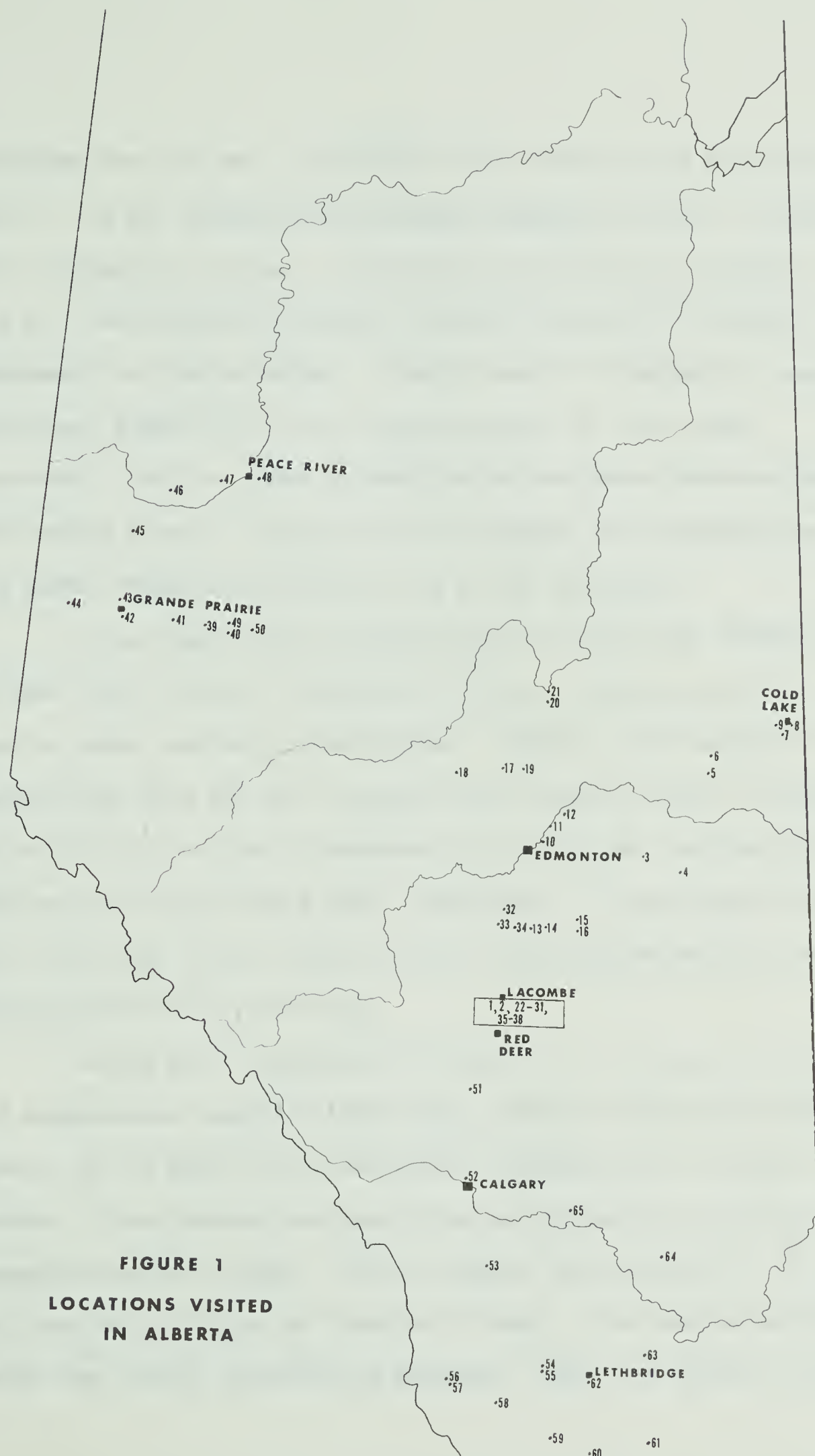
NO.	LOCATION	DESCRIPTION	DATE	NO.	LOCATION	DESCRIPTION	DATE
1	Barnett Lake	L	May 12	33	Pigeon Lake	L	June 9
2	Lacombe	D	May 12	34	Falun	D	June 9
3	Vegreville	D	May 18	35	Satinwood	S	June 9
4	Innisfree	P	May 18	36	Clive	P	June 9
5	Kehwin Lake	ST	May 18	37	Alix Lake	L	June 9
6	Sinking Lake	L	May 18	38	Mirror	P	June 9
7	Beaver River	R	May 18	39	Sturgeon Lake	L	June 13
8	Cold Lake	L	May 18	40	Valleyview	D	June 13
9	Cold Lake	D	May 18	41	Goose Creek	St	June 13
10	Bremnar	D	May 19	42	Grande Prairie	R	June 13
11	Fort Saskatchewan	St	May 19	43	Grande Prairie	D	June 13
12	Scotford	D	May 19	44	Beaver Lodge	P	June 13
13	Wetaskiwin	P	May 23	45	Rycroft	P	June 13
14	Gwynn	P	May 23	46	Fairview	P	June 13
15	Camrose	S	May 23	47	Grimshaw	D	June 13
16	Driedmeat Lake	L	May 23	48	Peace River	D	June 13
17	Westlock	D	May 26	49	Valleyview	D	June 14
18	Barrhead	D	May 26	50	Valleyview	St	June 14
19	Clyde	P	May 26	51	Olds	D	June 22
20	Collinton	St	May 26	52	Calgary	S	June 22
21	Athabasca	P	May 26	53	Nanton	S	June 22
22	Blindman River	R	May 30	54	Fort McLeod	R	June 23
23	Bentley	D	May 30	55	Fort McLeod	D	June 23
24	Bentley	P	May 30	56	Coleman	St	June 23
25	Eckville	P	May 30	57	Coleman	S	June 23
26	Eckville	D	May 30	58	Pincher Creek	P	June 23
27	Clive	P	May 30	59	Cardston	D	June 23
28	Clive	P	May 30	60	Del Bonito	S	June 23
29	Alix	P	May 30	61	Milk River	D	June 23
30	Mirror	D	May 30	62	Lethbridge	D	June 23
31	Mirror	P	May 30	63	Taber	D	June 23
32	Wizard Lake	L	June 9	64	Brooks	S	June 24
				65	Gleichen	S	June 24

NOTE: D-water filled ditch; L-lake; P-pond; R-river; S-slough; St-stream.









**FIGURE 1**  
**LOCATIONS VISITED**  
**IN ALBERTA**



organisms from the net, the jar was first filled to the brim with water. The net containing the trapped organisms was then inverted over the mouth of the jar. By pressing the nylon into the water in the jar, the organisms were able to swim off the net, relatively unharmed, into the container. A small amount of hydrophytic vegetation was added to the jar to supply oxygen for the trapped organisms. Care was taken to keep carnivorous forms separate from herbivorous forms. The jars were then capped for transportation and opened immediately upon arriving at the laboratory.

It was found that the coat-hanger net was sturdy enough to do some light probing in the debris and mud on the bottom of the pond or other area being investigated. However, to do extensive bottom sampling a dip net, purchased from a supply house, was used. One difficulty in using a purchased dip net was the fact that the mesh was too large to hold small crustaceans. It was found, however, that many insect larvae and other larger organisms could be obtained with this type of net.

During the investigation of many of the fresh-water habitats, mud samples were taken for later study. Many of the samples were taken from the edge of the area where it appeared that the water had receded. Some samples were taken from the dry bottoms of earlier temporary bodies of water. The mud samples were placed in 4 oz jars and labelled with date and location of sample. Mud samples were also taken from various areas during September, 1967, and February, 1968.



## CHAPTER III

### ANALYSIS OF TEACHER INTERVIEWS

The following analysis is the result of a detailed study of the interview sheets administered to the eleven biology teachers of the County of Lacombe.

Question 1. "What organisms do you collect locally and make use of with your classes in the teaching of biological concepts?"

The teachers were asked to list all local animal organisms that they had used in their teaching. The organisms listed were then classified according to phyla (Figure 2).

Only two phyla, Protozoa and Arthropoda, appeared to have been used to any great extent. On the basis of information obtained from this question, it was not possible to identify the organisms beyond the phylum level for the protozoans. However, the teachers interviewed all gave the common names of the arthropods used. Therefore it was possible to analyze the Phylum Arthropoda further by doing an analysis according to class (Figure 3).

Question 2. "Where and how do you obtain these organisms?"

Thirty-six percent of the teachers stated that no organisms were collected, by either the teacher or students. The remaining sixty-four percent of the teachers stated that the organisms were brought into the classroom by the students. In only eighteen percent of the cases were the teachers actually involved in the collection of



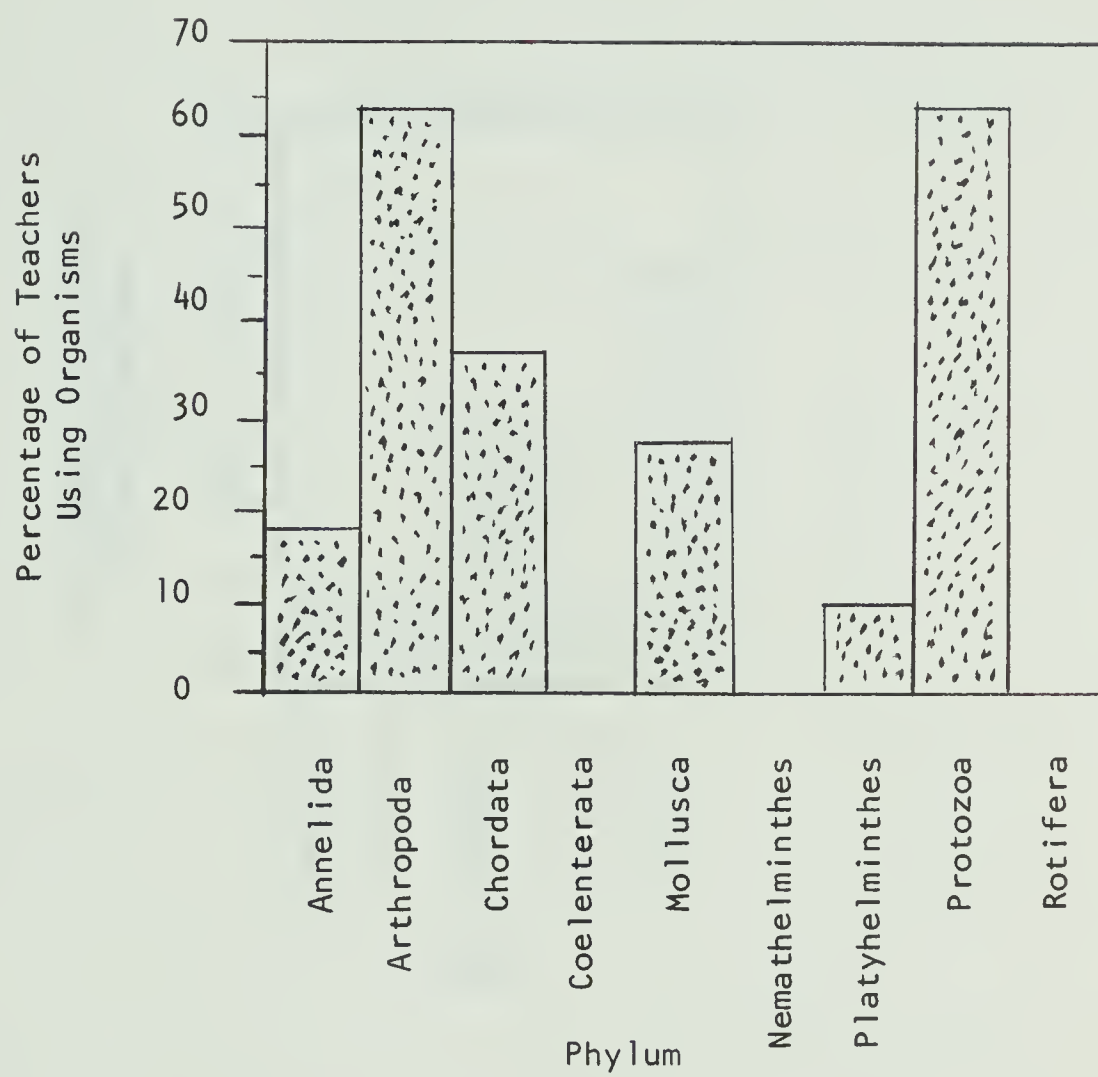


FIGURE 2

PERCENTAGE OF TEACHERS USING  
VARIOUS PHyla OF ORGANISMS





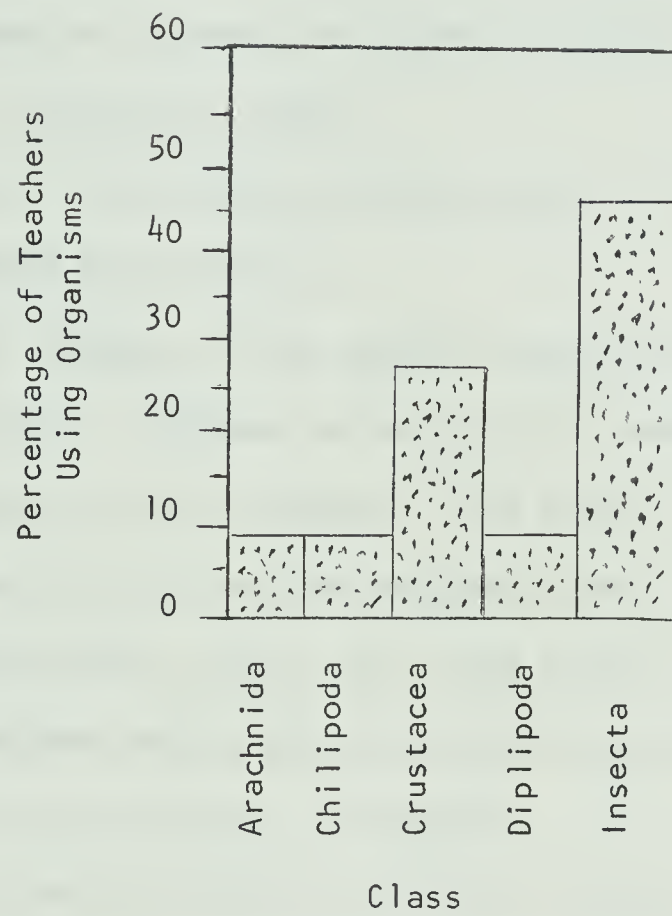


FIGURE 3

PERCENTAGE OF TEACHERS USING VARIOUS  
CLASSES OF ARTHROPODS



organisms. These results would seem to indicate that the students have an interest in collecting living specimens, but that only a small number of teachers were involved in any type of collecting activity. In almost all cases, the students collected the organisms from local ponds, creeks, and lakes.

Question 3. "List those organisms which you culture for study. What methods do you use?"

Sixty-four percent of the teachers stated that they did not culture any organisms. Eighteen percent of the teachers cultured Paramecium by means of a hay infusion. Nine percent cultured crustaceans and snails by obtaining pond water and letting it stand in sunlight. Nine percent had an ant or bee colony for observation purposes. Nine percent had an aquarium, which contained tropical fish, and a terrarium containing salamanders. It is interesting to note that most of the culturing was done by a relatively small number of teachers (thirty-six percent). Of the teachers who did culture any organisms, half or fifty percent cultured only one organism, such as Paramecium. Only one teacher, twenty-five percent of those who cultured any organisms, cultured at least four different kinds. Of the total number of teachers involved in the study, only nine percent cultured at least four different kinds of organisms.

Question 4. "How do you use these organisms which you collect locally or culture?"

Thirty-six percent of the teachers did not collect or culture



any organisms, therefore the question did not apply to them. The teachers who did use the materials had some agreement on only three points of usefulness. Seventy-one percent used live organisms to help teach classification. Fifty-seven percent used live organisms as a means of introducing work with the microscope. Twenty-eight percent thought that the manipulation of materials was an important aspect. The rest of the responses to the question were quite varied. The topics for which the living materials were used included: life cycles of insects, introduction to cells, awareness of local environment, training in observation, examples of animal movement, anatomy, and stimulation of interest in the students. Again, it is interesting to note that of all the teachers who did use local organisms as teaching aids, forty-three percent used them in only one of the above aspects, or for only one reason. Only fourteen percent of the teachers using the organisms listed four ways in which the organisms were used. This means that of the total number of teachers in the sample, only nine percent used the organisms to help teach at least four different biological concepts or skills.

Question 5. "What do you visualize as the major reasons why teachers should use local resources?"

The majority of teachers interviewed, sixty-four percent, stated that the main reason for using local materials was to stimulate interest and motivation in the students. Table III lists the reasons given for using local resources and the frequency of the responses.



TABLE III

## REASONS GIVEN FOR USING LOCAL RESOURCES

REASON GIVEN	TEACHERS GIVING REASON PERCENT	NUMBER
1. To stimulate student interest and motivation	64	7
2. To develop an appreciation of the local environment	36	4
3. To have more "meaningful" teaching.	27	3
4. To illustrate taxonomy.	9	1
5. To stimulate student involvement.	9	1
6. To observe the organisms first hand.	9	1
7. Economical.	9	1
8. To build a permanent collection.	9	1
9. To illustrate agricultural controls.	9	1
10. To have "better teaching" by means of a laboratory approach.	9	1





One teacher stated that it is too inefficient and time consuming to use local resources and, therefore, did not think teachers should use them. Also, one teacher did not think it mattered whether local organisms or specimens obtained from supply houses were used, as long as their use satisfied a definite purpose.

Question 6. "What do you visualize as the major reasons why teachers may not use local resources extensively?"

There appeared to be some general agreement among the teachers interviewed that the time spent in collecting organisms can be better utilized for other purposes, although the other purposes were not specifically outlined. Also, many teachers agreed that lack of knowledge of what organisms exist in the local environment, where they can be found, and methods of obtaining and culturing them are prime factors influencing the limited use of local resources. Table IV lists the reasons given for not using local resources and the frequency of the response.

## DISCUSSION

Of the teachers interviewed, thirty-six percent did not collect or use any local organisms. Organisms from nine phyla were included in the study and of these, three, the Coelenterata, Nematelminthes, and Rotifera, were not used or collected by any of the teachers. By comparing the responses given in question three (List those organisms which you culture for study.), with the



TABLE IV

## REASONS GIVEN FOR NOT USING LOCAL RESOURCES

REASON GIVEN	TEACHERS GIVING REASON PERCENT	NUMBER
1. Too time consuming.	63	7
2. Inadequate knowledge of what organisms exist locally.	63	7
3. Inadequate knowledge of where organisms can be found.	63	7
4. Inadequate knowledge of culturing techniques.	63	7
5. Inadequate knowledge of how to obtain living specimens.	54	6
6. Inadequate physical facilities for collecting and culturing.	45	5
7. Inadequate teacher background and training.	27	3
8. Organisms not present year-round.	18	2
9. Organisms not present in some localities.	9	1
10. Inconvenient.	9	1
11. Difficult to culture.	9	1
12. Lack of student interest.	9	1



responses given for question one (What organisms do you collect locally and use with your classes in the teaching of biological concepts?), it was possible to determine to some extent the numbers and kinds of organisms of the various phyla which were used or cultured.

#### Phylum Annelida

Eighteen percent of the teachers stated that annelids were collected. Analysis of the interview showed that earthworms were used by two teachers, although neither teacher cultured earthworms. Other annelids, such as leeches or oligochaetes were not used.

#### Phylum Arthropoda

Sixty-three percent of the teachers stated that they collected and used arthropods. It was found that one teacher used a spider; one, a centipede; and one, a millipede. Three teachers used crustaceans, usually Daphnia sp., and five used insects. The most commonly used insects were ants, bees, wasps, flies, butterflies, and moths. None of the teachers used all six kinds of these insects, and more often than not, only one kind of insect per teacher was used. No collecting of aquatic insects or aquatic insect larvae was carried out by any of the teachers. Only one teacher cultured arthropods, and that involved maintaining both an ant and a bee colony for observation purposes.



### Phylum Chordata

Thirty-six percent of the teachers collected and used chordates. One teacher used a field mouse and one a garter snake. Another teacher stated that he used field mice, gophers, and salamanders. The fourth used a garter snake, frogs and minnows. Only one teacher cultured any of the chordates, and these were frogs and salamanders.

### Phylum Mollusca

Three teachers, or twenty-seven percent, collected molluscs. All the samples collected and used were snails. No teachers collected and used freshwater clams. Of the three teachers using snails only one stated that the organisms were cultured.

### Phylum Platyhelminthes

One teacher of the eleven interviewed stated that planarians were collected. However, no culturing of the specimens was attempted.

### Phylum Protozoa

Sixty-three percent of the teachers stated that they collected and used protozoans. It seems apparent that the most commonly used protozoan was Paramecium, which was obtained from local ponds or cultured by means of a hay infusion. No mention was made of Amoeba, Stentor, or any other common protozoan. It is worth noting that if the teachers were using hay infusions or pond cultures, it is almost







certain that protozoans other than Paramecium were present. For example, one would likely find rotifers and nematodes in the same culture. One can only speculate that these organisms were probably present but that teachers and students either did not recognize them or did not spend much time investigating the samples. Only two teachers cultured protozoans, both Paramecium and by means of hay infusions.

It must be mentioned that the graphs (Figure 2 and Figure 3) have a major short-coming. Any organisms which teachers stated they used were included in the survey. Consequently, the amount of time spent using the organism, or the number of times that it was used is not shown. It is the writer's opinion that most organisms were used rather infrequently and certainly not to maximum advantage.



## CHAPTER IV

### AVAILABILITY AND DISTRIBUTION OF ORGANISMS

#### INTRODUCTION

The organisms to be considered in this chapter were collected from May 12 to June 24, 1967. Locations from which the samples were taken are plotted on a map of Alberta (Figure 1), and are also listed in Table V. On the average, a period of only fifteen to twenty minutes was spent collecting at each location. Consequently, the organisms which were found were quite plentiful and readily accessible. Most areas were investigated at shallow depths. Undoubtedly, many organisms would be found much more often than they appear in the table if a more extensive investigation of any given location were done. Only macroscopic organisms are listed in the table, although protozoans and rotifers are probably found in all areas (Pennak, 1953). No extensive bottom sampling of the locations was carried out. It is the author's opinion that other organisms, such as Tubifex, other annelids and midge larvae were much more plentiful than Table V would suggest. It must be noted that Table V is not a table of abundance of organisms. No attempt was made to correlate numbers of organisms found with locations sampled.

#### DISCUSSION

The areas investigated can be divided into two major



TABLE V

## ORGANISMS FOUND AT SPECIFIC LOCATIONS

No.	LOCATION	DESCRIP- TION	DATE	NEMATODES	GORDIANS	TURBELLARIANS	LEECHES	OLIGOCHAETES	SNAILS	CLAMS	HYDRA	FAIRY SHRIMPS	CLADOCERANS	AMPHIPODS	COPEPODS	OSTRACODS	WATER MITES	BEETLE LARVAE	BEETLE ADULTS	SPRINGTAILS	MOSQUITO LARVAE	MOSQUITO PUPAE	PHANTOM MIDGE LARVAE	TRUE MIDGE LARVAE	MAYFLY LARVAE	WATER STRIDERS	WATER BUGS	ORAGONFLY LARVAE	DAMSELFLY LARVAE	STONEFLY LARVAE	CADDISFLY LARVAE	AMPHIBIANS
1	Barnett Lake	L	May 12						X					X			X		X		X											
2	Lacombe	D	May 12																X													
3	Vegreville	D	May 18																X													
4	Innisfree	P	May 18																X													
5	Kehwin Lake	St	May 18																X													
6	Sinking Lake (Saline)	L	May 18																X													
7	Beaver River	R	May 18																X													
8	Cold Lake (Iced)	L	May 18																X													
9	Cold Lake	D	May 18																X													
10	Bremner	D	May 19																X													
11	Fort Saskatchewan	St	May 19																X													
12	Scotford	D	May 19																X													
13	Wetaskiwin	P	May 23	X															X													
14	Gwynn	P	May 23																X													
15	Camrose	S	May 23																X													
16	Driedmeat Lake	L	May 23																X													
17	Westlock	D	May 26																X													
18	Barrhead	D	May 26																X													
19	Clyde	P	May 26																X													
20	Collinton	St	May 26		X														X													
21	Athabasca	P	May 26																X													
22	Blindman River	R	May 30																X													
23	Bentley	D	May 30																X													
24	Bentley	P	May 30																X													
25	Eckville	P	May 30																X													
26	Eckville	D	May 30																X													
27	Clive	P	May 30																X													
28	Clive	P	May 30																X													
29	Alix	P	May 30																X													
30	Mirror	D	May 30																X													
31	Mirror	P	May 30																X													
32	Wizard Lake	L	June 9																X													

D-water filled ditch; L-lake; P-pond; R-river; S-slough; St-stream. X-denotes organisms found at location.





TABLE V (continued)

No.	LOCATION	DESCRIP- TION	DATE	NEMATODES	GORDIANS	TURBELLARIANS	LEECHES	OLIGOCHAETES	SNAILS	CLAMS	HYDRA	FAIRY SHRIMPS	CLADOCERANS	AMPHIPODS	COPEPODS	OSTRACODS	WATER MITES	BEEBLE LARVAE	BEEBLE ADULTS	SPRINGTAILS	MOSQUITO LARVAE	MOSQUITO PUPAE	PHANTOM MIDGE LARVAE	TRUE MIDGE LARVAE	MAYFLY LARVAE	WATER STRIDERS	WATER BUGS	DRAGONFLY LARVAE	DAMSELFLY LARVAE	STONEFLY LARVAE	CADDISFLY LARVAE	AMPHIBIANS
33	Pigeon Lake	L	June 9																													
34	Falun	D	June 9																													
35	Satinwood	S	June 9																													
36	Clive	P	June 9																													
37	Alix Lake	L	June 9																													
38	Mirror	P	June 9																													
39	Sturgeon Lake	L	June 13																													
40	Valleyview	D	June 13																													
41	Goose Creek	St	June 13																													
42	Grande Prairie	R	June 13																													
43	Grande Prairie	D	June 13																													
44	Beaverlodge	P	June 13																													
45	Rycroft	P	June 13																													
46	Fairview	P	June 13																													
47	Grimshaw	D	June 13																													
48	Peace River	D	June 13																													
49	Valleyview	D	June 14																													
50	Valleyview	St	June 14																													
51	Olds	D	June 22																													
52	Calgary	S	June 22																													
53	Nanton	S	June 22																													
54	Fort McLeod	R	June 23																													
55	Fort McLeod	D	June 23																													
56	Coleman	St	June 23																													
57	Coleman	S	June 23																													
58	Pincher Creek	P	June 23																													
59	Cardston	D	June 23																													
60	Del Bonito	S	June 23																													
61	Milk River	D	June 23																													
62	Lethbridge	D	June 23																													
63	Taber	D	June 23																													
64	Brooks	S	June 23																													
65	Gleichen	S	June 24																													

D=water filled ditch; L=lake; P=pond; R=river; S=slough; St=stream. X=denotes organisms found at location.





categories; lotic and lentic waters. The lotic waters will include both streams and rivers because of the relatively small number investigated. The lentic waters are divided into four sub-categories including lakes, ponds, water filled ditches, and sloughs. Of the sixty-five locations investigated, thirty-four percent were water filled ditches; twenty-six percent, ponds; twelve percent, lakes; eleven percent, sloughs; and seventeen percent lotic water (eleven percent streams and six percent rivers).

The Province of Alberta was divided into three geographic areas by lines of demarcation drawn east and west through Edmonton and Calgary. Areas north of Edmonton were considered as northern Alberta; areas between Edmonton and Calgary, central Alberta; and areas south of Calgary, southern Alberta.

#### Phylum Porifera

During the investigation no freshwater sponges were found. However, during a subsequent investigation during July, 1968, freshwater sponges of the family Spongillidae were found in central and northern Alberta. One specimen was taken from a slow moving stream in northern Alberta, another from a slough in central Alberta.

#### Phylum Coelenterata

The only members of this phylum found were hydra, and they were found in nine percent of the areas investigated. During the investigation period hydra were found in all categories of water except



lakes. Hydra were, however, found in Lake Wabamun during a later investigation which took place during February, 1968. The animals were most commonly found attached to submerged vegetation or rocks. Hydra were located in north, south, and central Alberta. The hydra appeared to be more common during the late spring and early summer, although they have been obtained from the outlet canal of the power plant located on the north shore of Lake Wabamun, during February and March.

#### Phylum Platyhelminthes

The free-living members of this phylum, Class Turbellaria, were found in six percent of the areas investigated. During the time of investigation, they were found in all categories of water environments except lotic. However, during subsequent investigations, planaria have been found in the outlet canal from the power plant on Lake Wabamun. The water in the canal is moving and would have to be classed as lotic.

The type of environment determined to a great extent the order of turbellarian found. If the water was standing and appeared to have a limited oxygen supply, members of the Order Rhabdocoela were most commonly found. If the water was relatively fresh and contained a good supply of oxygen, such as in lakes or streams, members of the Order Tricladida were found. The rhabdocoels were most often found attached to submerged vegetation or along the mud bottom. The tricladids were most often found attached to submerged rocks or



vegetation. Planarians were found in north and central Alberta.

#### Phylum Nemathelminthes

Members of the Class Nematoda were found in only six percent of the areas investigated. However, according to Pennak (1953), almost any collection of sand, mud, debris, or vegetation from the bottom of an area containing water will yield nematodes, usually less than one centimeter long. The reason that nematodes were not found frequently was undoubtedly due to the very brief investigation of the bottoms of areas visited. In the author's opinion, nematodes are much more abundant than the conducted survey would appear to indicate. Nematodes were found in central and southern Alberta, and in all types of water except lakes and ditches.

#### Phylum Nematomorpha

Only one gordian worm was found during May and June, 1967. The specimen was found in a very small stream near Collinton in northern Alberta. According to Pennak (1953), very little is known about gordian ecology.

One other specimen was obtained at a later date. A caddis fly larva was obtained from Lake Wabamun in February, 1968. The larva was placed in an aquarium without the presence of any other large organisms, and a short time later the gordian worm was found to be present in the tank. The most likely explanation for the presence of the worm is that the larva had parasitized the caddis fly larva and later





emerged as an adult gordian.

### Phylum Annelida

Members of the Class Hirudinea were found in twenty percent of the areas investigated and were found in all categories of water environments in north, south, and central Alberta. Members of the Order Rhynchobdellida were found with the largest number of specimens falling into the Family Glossiphoniidae. They were found most often in standing water attached to vegetation and many times were carrying young on their ventral surface. Members of the Order Arhynchobdellida were also found with specimens from the Family Hirudiinae being most common. Occasionally these specimens could be seen swimming freely in the water, but more often they are found on the bottom.

Leech egg cases, light brown, oval, and approximately one-half centimeter long, were found in many areas throughout the province. They were most often found attached to submerged rocks.

Members from the Class Oligochaeta were found in twelve percent of the areas investigated and were found in all major regions investigated. During May and June 1967, specimens were taken from all types of water except lakes and sloughs. However, on subsequent investigations Tubifex sp. have been located in Lake Wabamun and also a slough located near Edmonton. Therefore, it seems safe to state that oligochaetes can be found in all categories of water environment.





The most commonly found specimens were members of the genus Tubifex. According to Pennak (1953), members of this genus are very common and widespread throughout the United States. In the author's opinion, a more comprehensive survey of bottom samples would probable indicate a much higher percentage of annelids present than the survey indicates.

#### Phylum Mollusca

Of the Class Gastropoda, the Order Pulmonata was widespread throughout the province, being found in north, south, and central Alberta. Snails were found in forty-two percent of the areas investigated and were located in all categories of water. The most commonly found specimens belonged to the Family Lymnaeidae, which can be recognized by the spiral and dextral nature of the shell. The Family Physidae with the spiral and sinistral shell were also found. Members of the Family Planorbidae were also found, but not as frequently as the Physa and Lymnaea. The two most commonly found orb snails were of the genus Helisoma and Gyrulus, which can be recognized by the flat patelliform shape of the shell.

The Class Pelecypoda was not found as often as the Class Gastropoda. Clams were found in only eight percent of the areas investigated. Pennak (1953) states that freshwater bivalves are most abundant in larger rivers. Of the rivers investigated, fifty percent were found to contain bivalves. The only members found in lentic water were the Family Sphaeriidae, the fingernail clams. Pelecypods were found to be widely distributed in north, south, and central Alberta.



## Phylum Arthropoda

Approximately seventy percent of the organisms listed in Table V fall into the Phylum Arthropoda. Because of this, and for ease of discussion, this phylum is divided into classes and orders.

### Class Crustacea

#### Sub-class Branchiopoda

Two orders, Anostraca and Cladocera, were found in this sub-class. The order Anostraca is comprised of various species of fairy shrimps. These organisms were found in fifteen percent of the areas investigated and were located in central and northern Alberta. May 30 was the last date fairy shrimps were found. No investigation was made of areas located in southern Alberta until June. It is likely that fairy shrimps were present in southern Alberta during late April and early May. Fairy shrimps were found in all categories of aquatic environment except lakes. It was found that they achieved their greatest densities in ponds, sloughs, and roadside ditches. Pennak (1953) states that fairy shrimps are only rarely located in bodies of water exceeding one acre in area.

Cladocerans were found throughout the summer months, although none were found as early in the year as were fairy shrimps. Cladocerans are both widespread and numerous, with specimens found in north, south, and central Alberta and in sixty-eight percent of the locations investigated. Cladocerans were found in all categories



of aquatic environments.

It was brought to the author's attention that tadpole shrimp, Apus sp., a member of the order Notostraca, had been found in a roadside pond near Vegreville in central Alberta during June, 1968. During the author's field investigations no members of this order were found.

#### Sub-class Copepoda

Copepods were found in forty-two percent of the areas investigated and were widespread, being found in north, south, and central Alberta, and in all categories of aquatic environment. The most commonly collected organisms were of the sub-orders Calanoida and Cyclopoida. Pennak (1953) states that the most common copepods present in the United States are Cyclops sp., and Diaptomus sp.. Members of both genera were found in Alberta.

#### Sub-class Malacostraca

Members of the Order Amphipoda were found in thirty-four percent of the areas investigated. They were found in all categories of habitat in north, south, and central Alberta. The two most commonly found freshwater shrimps were Gammarus sp. and Hyalella sp.. Pennak (1953) states that amphipods serve as intermediate hosts for a wide variety of parasites.





### Sub-class Ostracoda

Ostracods were found in thirty-eight percent of all areas visited and were found in all types of aquatic environments. They were widely distributed and were found throughout the province. Most specimens are located on or near the bottom in shallow water. Identification of species is difficult and Pennak (1953) states that little investigation of ostracods has been done.

### Class Arnachnida

Most aquatic members of this class belong to the Order Hydracarina, commonly known as the water mites. Specimens were found in fifty-one percent of the field locations throughout the province. They did not appear to be restricted to habitat as they were found in all types of aquatic environments. Identification to species is difficult and Pennak (1953) gives a key to genera only.

### Class Insecta

#### Order Coleoptera

Water beetle larvae and adults were found throughout the province with members of the Dytiscidae family being the most common. Beetle larvae were found in twenty-eight percent of the locations investigated, while the adults were found in thirty-seven percent. Adults were found in all categories of aquatic environment, but the larvae appeared limited to lentic waters. According to Pennak (1953) larvae are also found in lotic waters, and the relatively small number





of rivers and streams investigated is probably the main reason why larvae were not found in these locations.

#### Order Collembola

Springtails were found throughout the province in all types of water environment. They were collected from twenty-nine percent of the areas visited. The springtails are not truly aquatic as they never purposely submerge. They were most often found near the shore floating on the surface film of the water.

#### Order Diptera

None of the adult dipterans are truly aquatic, but many species have aquatic larval stages. The Culicidae family includes both mosquitoes and phantom midges. Mosquito larvae (Culex sp.) were found in thirty-one percent of the areas investigated and in all water categories except lakes, although Pennak (1953) states that mosquito larvae are often found in large bodies of water. The pupae were found in fourteen percent of the areas investigated, but none were found in lotic waters or lakes. The pupa stage lasts for several days while the larval stage may last a month or more. Because of this, the larvae appear more common than the pupae. The earliest sighting of a pupa in central Alberta was May 30, 1967, approximately one month after the first sighting of larvae. Both larvae and pupae were found throughout the province.



The phantom midge larvae (Chaoborus sp.) were also found throughout the province. Larvae were found in seventeen percent of the areas visited and in all water categories except lakes, although Pennak (1953) states that Chaoborus sp. is often found in lakes. As with the mosquito, the pupa of the phantom midge is short lived and will appear to be less common than the larva.

True midge larvae, Family Tendipedidae, were found throughout the province with Chironominae being the most common sub-family. Larvae were found in fourteen percent of the areas investigated, but because the majority of the larvae are bottom dwellers, the organisms are undoubtedly more common than the survey would appear to indicate. The pupa resembles the pupa of the Culicidae superficially, and appeared more often late in spring and early summer. Because of the late time of development, midge pupae were not found and are therefore not listed in the table. Midge larvae were found in all categories of water except lotic, but Pennak (1953) states that there are lotic dwelling species as well.

During July, 1968, a slowly drying water filled ditch was found in central Alberta. Here, in damp muddy areas with no water present, Chironomous sp. larvae were found in great numbers with relative ease by simply digging into the surface layers of mud.

#### Order Ephemeroptera

Mayfly larvae were found throughout the province in all



categories of water. Seventeen percent of the areas visited were found to be the habitat of the larvae. Members of this order may be found climbing on vegetation, crawling on the bottom, or burrowing in the mud.

#### Order Hemiptera

The true water bugs are a rather widespread group. The water striders, Family Gerridae, were found in twenty-nine percent of the areas investigated throughout Alberta. They were found in all categories of water except lakes, although subsequent investigations have shown them to be present near the shores of Lake Wabamun in central Alberta.

Either water boatman (Family Corixidae) and backswimmers (Family Notonectidae), or both, were found in thirty-one percent of the areas investigated. Members of these two families were found throughout the province and in all categories of water.

#### Order Odonata

The true dragonfly larvae, Sub-order Anisoptera, were found in nine percent of the areas investigated. Members were found throughout the province but appeared confined to water-filled ditches, ponds, and sloughs. Pennak (1953) states the larvae may be found in streams and the shallows of lakes.

Damselfly larvae, Sub-order Zygoptera, were more common than dragonfly larvae being found in eighteen percent of the areas





investigated. They were found in all types of aquatic environment except lakes, but again Pennak (1953) mentions that they may be present in the shallows of lakes. The larvae were widespread being found throughout the province.

#### Order Plecoptera

Stonefly larvae were found in eight percent of the areas investigated, but were almost invariably in lotic waters as none were found in ponds or water filled ditches. They appeared to be widespread and were found throughout the province. Specimens were most often found on the bottom hidden under submerged rocks.

#### Order Trichoptera

Caddis fly larvae were quite plentiful and were found in forty-nine percent of the areas investigated. Many different species were found throughout the province. Caddis flies were found in all types of aquatic environment and on all types of substrates. However, Pennak (1953) states that some larvae, especially naked caddis larvae, do have preferential habitats.

### Phylum Vertebrata

#### Class Amphibia

Adult amphibians, frogs and toads, were found throughout the province in thirty-eight percent of the localities visited. They were found in all categories of habitat, except lakes, but subsequent





investigations have shown organisms to be present in Lake Wabamun. Tadpoles of frogs and toads were found in all categories of water throughout the province.

On July 4, 1968, a tiger salamander was collected from a slough in central Alberta. During the investigation in 1967, no salamanders were found.

#### Class Osteichthyes

Members of this class have been omitted from Table V because no attempt was made to collect specimens. However, during previous and subsequent investigations, sticklebacks, spottail shiners, Johnny darters, dace and young pike, perch, and suckers were obtained from lakes and streams in central and northern Alberta. Many specimens, especially sticklebacks, are an excellent source of tapeworm larvae as the fish serve as intermediate hosts for many waterfowl tapeworms and flukes.

#### SUMMARY

It was found that all of the aquatic environments studied contained animal life of some type. Water filled ditches, ponds, and sloughs seemed to have the largest variety of fauna of the six categories investigated. Only a small number of organisms, such as fairy shrimps, seemed to have seasonal limits within which they can be collected successfully.



It was found that weather influences the collection of many organisms. During cold, cloudy days, when the air temperature was about fifty degrees Fahrenheit or less, crustaceans, water bugs, beetles, and insect larvae appeared to be less active than on warm, clear days. During windy days when the surface of the water was disturbed, surface dwelling organisms, along with many of the smaller sub-surface organisms, proved relatively scarce in collections. If a location, relatively poor in numbers of organisms present on a windy day, was re-visited on a calm day many more specimens were found.

- It seems likely that a more comprehensive survey might well reveal the presence of most of the organisms listed in Table V, on a province-wide basis. With a minimal amount of time and effort, one should be able to find a great many water-dwelling organisms in most areas of the province throughout the spring and fall months when schools are in session.



## CHAPTER V

### CULTURING OF COLLECTED SPECIMENS

The best culture methods for maintaining living organisms are those that reproduce the most favorable environment and eliminate natural enemies (Brandwein, 1966). There are a few general conditions that should be kept in mind whenever an attempt is being made to culture many aquatic organisms.

1. Try to maintain the culture in the temperature range between 18-21 degrees Centigrade.
2. Keep the culture away from the fumes of volatile substances.
3. Keep cultures approximately neutral ( $p^H$  of 7.0).
4. Keep the cultures in medium light.
5. Avoid drafts which can cause temperature fluctuations or carry airborne contamination.
6. Keep the glassware clean at all times (Brandwein, 1966).

#### Phylum Protozoa

The protozoans are a versatile and easily cultured phylum. Members of the classes Sarcodina, Ciliata and Mastigophora were cultured successfully. Numerous references list culturing techniques for the protozoans (Appendix III).

A successful culture method used in this study was that of a hay infusion. The hay was obtained from a field adjacent to a pond near the town of Westlock in northern Alberta on May 29, 1967.



Several stalks of hay were placed into each of three 32 oz jars. The jars were then filled three-quarters full with Brandwein's solution, Chalkey's solution, and aged tap water respectively (Appendix IV). Within three days, the water in all jars had turned cloudy and a scum had begun to form on the surface. At that time, no macroscopic organisms were found in any of the cultures. By June 5, all three jars contained a number of protozoans, including amœebas, ciliates, and flagellates in the scum near the water's surface. Bacteria were also present in the cultures. Brandwein's solution appeared to have less scum on the surface than the remaining two cultures, but contained a more concentrated culture of protozoans. Organisms were kept alive in all three cultures for a four-month period with an occasional addition of water or solution as necessary. When the numbers of organisms appeared to decrease, a small amount of hard-boiled egg yolk paste added to the culture, about once every two weeks, restored the number of organisms (Appendix IV).

If a pure culture of one type of organism is desired, there are several methods one can use (Appendix III). In all cases, the desired organism must be inoculated by carefully pipetting them into a sterile culture medium. In a pure culture, supplementary food such as rice or wheat grains will need to be added periodically.

#### Phylum Coelenterata

Attempts made to culture hydra in 32 oz jars met with varying







degrees of success. Four cultures of hydra were started in pond water from June 9 to June 22, 1967. Three cultures contained living hydra for approximately one month, while the remaining culture remained successful from June 9 to October 3. At that time, the hydra went into a period of depression and died.

It was found that hydra need a good supply of oxygen, require careful attention to feeding, and survive longer in cultures which do not exceed room temperature. Cultures were kept in indirect light to prevent temperature fluctuations due to direct sunlight. The three cultures which were successful for approximately one month were fed a mixture of cladocerans and copepods about every second day. If food was present in the culture, the hydra did not receive additional crustaceans until their food supply was noticeably diminished.

The fourth culture was handled very differently. The hydra were collected from a ditch near Falun in central Alberta on June 9, 1967. A culture of crustaceans was started in water from the ditch and hydra were added to the culture. Pond weed (Potamogeton sp.) was added to supply the culture with oxygen. The culture was then treated as a culture of crustaceans with no special care being given to the hydra. The culture lasted successfully for approximately four months, at which time the hydra died. The crustaceans continued to live.

The cultures appeared to go through a stage of rapid growth with budding and sexual differentiation taking place soon after the



cultures were started, often as soon as three or four days. There was no slow deterioration of the cultures. When the hydra died, they did so rapidly and over a short period of 2-3 days. Death of the culture was always preceded by a period of depression of the hydra in which the body and tentacles would contract into a small ball. Aged tap water was added to the cultures as needed, and it may be that too much water was added at one time. Perhaps this prevented the hydra from becoming acclimated to the changing water and therefore brought about the death of the organism.

#### Phylum Platyhelminthes

Both orders, Rhabdocoela and Tricladida, were cultured. Two cultures of rhabdocoels were maintained, one lasting two weeks, another lasting six weeks. The planarians were obtained from a pond near Mirror in central Alberta on June 9, 1967. Six were placed in a 32 oz jar containing pond water. Six more were placed in pond water in a large battery jar containing vegetation and two inches of mud sediment. Both cultures were shaded to prevent direct sunlight from reaching the cultures.

Both cultures were fed raw liver every third day. At no time were the planarians found to be feeding. Most of the uneaten liver was removed one hour after it had been placed in the culture. By June 16, the water in the 32 oz jar had become fouled by the excess raw liver. The planarians were then transferred to a large fingerbowl



(8 $\frac{1}{4}$  inch diameter) containing Chalkey's solution (Appendix IV), and a few sprigs of Elodea. The batter jar contained a snail, Lymnaea, which fed on the excess meat. The water in the battery jar did not become fouled.

When the rhabdocoels were transferred to the fingerbowl, they became very active. Approximately one-half hour after the transfer, one planarian gave birth to five live young. The parent planarian appeared to go into convulsions during birth. The body was curled until the anterior and posterior ends met. The body was then flexed and straightened in a rapid movement. At this time a young planarian was set free from the parent. This process was witnessed five times during a period of approximately fifteen minutes, after which the parent continued to live. The young were miniature adults, approximately one and one-half millimeters long.

One June 16, following the birth of the young planarians, crustaceans were added to the rhabdocoel culture. Within a short period of time, one of the mature planarians had seized a cladoceran and fed on the organism. During the next several hours, all of the adult planarians except the one which gave birth had fed at least once.

On June 17, five tricladids from Wizard Lake were added to the rhabdocoel culture. Within one hour, a tricladid had captured and devoured a rhabdocoel. By June 26, all rhabdocoels in the culture had been eaten by the tricladids. During feeding, the tricladids had





devoured the entire organism except for the thick-shelled eggs which were present within the body of the rhabdocoels. Under normal circumstances, these eggs are not laid by the planarians. They are freed from the parent's body only when it dies (Engelhardt, 1964). Because of the predatory habits of the tricladids, it is not wise to try to raise a mixed culture of tricladids and rhabdocoels.

The rhabdocoels which were cultured in the battery jar appeared to thrive. Crustaceans were used as a food supply. The planarians appeared to remain on or near the bottom of the jar for six weeks. After that time, they were not seen. They may have burrowed into the mud but no concrete evidence of this was found.

Three cultures of tricladids were maintained. Five tricladids from Wizard Lake were added to the rhabdocoel culture on June 17, 1967. They were fed raw liver every two days. The excess liver was removed as soon as the organisms had stopped feeding. To avoid fouling the water the meat was removed before one hour had elapsed.

By June 21, tricladid egg cases were noted attached to the leaves and stalks of the Elodea in the culture. By June 28, eight egg cases had been produced. On June 27, the planarians, vegetation, and egg cases were transferred to a fresh fingerbowl of Chalkey's solution. Three of the five planarians died shortly after the transfer. By July 12, one of the egg cases had hatched and four young planarians, about two millimeters long, were present in the





culture. The planarians were again transferred to a fresh culture, this time all survived. By July 23, another egg case had hatched releasing three more planarians. On August 21, the last of the original planarians died leaving seven young planarians in the culture. The young remained living for another month at which time the culture was accidentally destroyed.

On September 12, the two cultures of tricladids from Lake Wabamun were started, one in a two-gallon aquarium and another in a 32 oz jar. Both cultures contained lake water and water crowfoot (Ranunculus sp.). The planarians were fed raw liver and hard-boiled egg yolk. The smaller planarians appeared to feed readily on the egg yolk and the branched gut could be clearly seen outlined in yellow. The larger planarians appeared to feed more readily on the liver. The aquarium culture contained approximately one hundred planarians and the majority remained alive for six weeks. The jar culture contained about twelve planarians which remained living for five months.

#### Phylum Nematelminthes

No special attempt was made to culture the free-living nematodes. However, various references outline culture methods (Appendix III). Probably the easiest is that of Pennak (1953). Prepare a culture of three percent agar in aged tap water, and add to this a small amount of soil and vegetal debris. This medium can



be used to culture herbivorous nematodes. However, some free-living nematodes are carnivorous and here such forms as crustaceans or rotifers would be necessary. Little is known about the longevity of nematodes (Pennak, 1953), therefore it is difficult to predict how long a successful culture could be maintained if breeding did not take place in the culture.

#### Phylum Nematomorpha

One adult gordian was collected and cultured. The organism was obtained on May 26, 1967 from a creek near Collinton in northern Alberta. It was placed in a 16 oz jar containing a small amount of vegetation and creek water. By June 5, the movements of the gordian had slowed noticeably. The gordian continued to live for another week at which time it was preserved in fifty percent alcohol. At no time was any food material introduced into the culture.

It is probable that the majority of gordians collected are males. The females are usually passive and are not as easily noticed as the males, which are very active. The mouth region of gordians is usually very small or degenerate, depending on the species (Pennak, 1953). It is therefore doubtful if supplementary feeding would extend the length of time a culture could be maintained successfully.

#### Phylum Annelida

##### Class Hirudinea

Cultures of both orders, Arhynchobdellida and Rhynchobdellida,



were maintained. Limited success was achieved with the Arhynchobdellida. Two leeches were placed in each of three 32 oz jars, two containing pond water and one containing Chalkey's solution. The specimens were obtained from Wizard Lake and Lake Wabamun on June 9 and September 12 respectively. The culture containing leeches from Wizard Lake in Chalkey's solution was fed raw liver, the other two containing leeches from Lake Wabamun were not fed. The leeches appeared to feed on the liver, but a large amount of meat residue remained. Because of this the water fouled and the leeches died within two weeks. The leeches in the remaining two cultures lived for approximately one month. Care was taken to see that the jars were loosely capped as leeches will often crawl out of the culture medium. Pennak (1953) states that leeches can be kept living in the laboratory for a period of up to two years if they have received a meal of whole blood prior to placing them in the culture.

Greater success was achieved in culturing leeches of the Order Rhynchobdellida. Three cultures, each containing two organisms, were maintained in 32 oz jars. One culture contained leeches obtained from a river near Fort McLeod on June 23, 1967. The culture medium was Chalkey's solution and a small amount of aquatic vegetation was present. One of the leeches added to the culture was carrying young attached to its ventral surface. One of the adults died in two months; the other, in three months. The young leeches remained living





until January 30, 1968, a period of seven months. No food materials were added to the culture at any time. Chalkey's solution was added to replace the water lost due to evaporation.

The remaining two cultures were maintained in lake water and creek water respectively. Distilled water was added when necessary to replace water lost. The leeches in the lake water lived for approximately four months, while those in creek water lived for eight months. Neither culture was fed. The leeches in lake water were much larger in size than the leeches in the creek water. It is the author's opinion that the smaller leeches were probably younger and this could account for the greater success of the culture.

#### Class Oligochaeta

Two cultures of Tubifex sp. were maintained. One culture was set up in a 32 oz jar containing lake water and mud sediment. The specimens were obtained from Lake Wabamun on September 12, 1967 and remained living until mid-November without the addition of supplementary food. The second culture was maintained in a battery jar containing pond water and mud sediment from a pond near Ellerslie in central Alberta, collected during October, 1967. To this culture was added, approximately every two weeks, a small amount of a paste made from hard-boiled egg yolk (Appendix IV). The organisms remained living for four months. Both cultures contained aquatic vegetation (Ranunculus sp.) and distilled water was added periodically to keep





the water level relatively constant.

One culture of bristleworms of the Family Naididae was maintained. The organisms were obtained from a ditch near Valleyview in northern Alberta on June 14, 1967. They were placed in a 32 oz jar containing some of the ditch water, vegetation, and some snails. Many bristleworms could be seen on the side of the container for two weeks. During the third week, the number of bristleworms began to diminish. By the end of the third week, no oligochaetes were found. No supplementary feeding was attempted.

#### Phylum Mollusca

It was found that successful cultures of the Order Gastropoda could be maintained with a minimum amount of effort. Ten cultures of mixed Lymnaea and Physa were maintained in 32 oz, 16 oz, and battery jars. The smaller jars contained two adult snails; the 32 oz jars, four; and the battery jar, twelve. Little difficulty was found maintaining the organisms at these concentrations.

The snails in the battery jar survived for three months. The cultures in the 32 and 16 oz jars were successfully maintained for periods ranging from three to eight months. No correlation was found between the size of the containers or the types of snails, Lymnaea or Physa, and the length of time the culture could be maintained successfully.

It is important to provide the snails with green vegetation,



such as water crowfoot (Ranunculus sp.), various species of pondweed (Potamogeton sp.), duckweed (Lemna sp.), or some type of green algae. If local hydrophytes are not available, fresh lettuce leaves added once a week may be substituted as a food supply.

It was found that the snails often laid eggs on the side of the containers or on the vegetation. These hatched liberating many young. Many of the cultures had a number of smaller snails present when the culture was terminated. Since all the snails were pulmonates, few difficulties were encountered from lack of oxygen present in the water. It is the author's opinion that, if desired, snails could probably be cultured in more concentrated numbers.

Two cultures were established using members of the Order Pelecypoda. The clams were obtained from Blind Man River near Lacombe on August 9, 1967. Five clams were placed in a two-gallon aquarium containing a fifty-fifty mixture of river and aged tap water. A mixture of one-third each of sand, mud, and gravel was used as a substrate in the tank.

A second culture, containing two clams, was set up in an established aquarium containing two Johnny darters and six longnosed dace. The tank was of two-gallon size and had a completely gravel substrate. Both cultures were fed pabulum, every second or third day, which was crushed very fine with a mortar and pestle. Both tanks contained an air-stone to insure an adequate supply of oxygen. The clams in the first culture remained living for four and one-half



months. The clams in the second culture, two months. According to Pennak (1953), clams are not often found on substrate composed of only gravel. This feature may have had an influencing factor on the longevity of the second clure.

If clams are being raised in a tank where an air-stone is not available, then only a small number of clams can be cultured. If no supplementary food is provided, only two or three clams per twenty gallon aquarium should be present (Turtox, 1944).

### Phylum Arthropoda

#### Class Crustacea

Most of the cultures of crustaceans were maintained in a similar fashion. The majority of cultures were established using 32 oz jars as containers with a variety of media, such as pond, ditch, and aged tap water, Chalkey's solution and Brandwein's solution (Appendix IV). A paste of hard-boiled egg yolk was added as food supplement to most cultures every three or four days.

It was found that a greater degree of success was achieved if a smaller number of organisms was used to begin the culture, about one or two organisms per ounce of water. If too many organisms were used at first, the culture usually died in a short time and the water fouled. When a smaller number of organisms was used, the culture soon reached an optimum level and remained about there.





There appeared to be a direct correlation between the amount of food added and the number of organisms present. The more egg yolk added, up to a point, the more concentrated the organisms appeared in the culture. It was noticed that supplementary feeding of crustaceans, especially the ostracods, not only increased the number of organisms present, but their size as well. Care must be taken as too much food can foul the water. Egg yolk was added as a food for five months, after which feeding of all cultures was stopped.

The jars used had a relatively narrow opening. If they were filled to the top the area of the water's surface exposed to the air was diminished. This prevented adequate gas exchange and many of the organisms died. To prevent this, the culture jars were filled only three-quarters full, or up to the base of the neck.

It was found that a small amount of aquatic vegetation added to the water appeared to enhance the success of the cultures. Filamentous algae, crowfoot (Ranunculus sp.), water milfoil (Myriophyllum sp.), and pondweed (Potamogeton sp.), seemed to be very good. A small amount of sediment, such as some of the substrate from the locality from which the specimens were taken, seemed useful in culturing most types of crustaceans. The cultures were placed in moderate light, without receiving direct sunlight for prolonged periods of time, because this could cause the temperature to fluctuate.





### Sub-Class Branchiopoda

Little success was achieved with the culturing of fairy shrimps (Eubbranchipus sp.). Several cultures were tried with the most successful lasting one week. Twelve shrimp were collected on May 30, 1967, and placed in an established two-gallon aquarium which did not contain any predators. The tank was kept shaded and a food supplement of hard-boiled egg paste was added daily. By June 2, over half of the organisms were dead, and on June 5, the last one died. Other cultures were tried using 32 and 16 oz jars with pond water or aged tap water as the medium. In all cases, the organisms survived less than one week.

According to Pennak (1953), many species of fairy shrimps do not survive well in temperatures exceeding fifteen degrees Centigrade. The temperature at which the cultures were maintained was approximately twenty to twenty-two degrees Centigrade. This temperature difference may be one of the reasons why little success was achieved with the cultures.

Several cultures of cladocerans were maintained, some with very good success. All of the cultures were in 32 oz jars, but a number of different types of water media were used. To keep the water level nearly constant, distilled water was added periodically to the cultures. However, in the case of those cultures containing aged tap water, only aged tap water was added.



Most of the cultures contained ostracods and copepods as well as cladocerans. The only time pure cultures of cladocerans were maintained was when the organisms were inoculated into a culture medium other than pond water. The other crustaceans present did not appear to have any adverse effects on the cladocerans, as there appeared to be no correlation between the success of the culture and whether the culture was pure or mixed.

One culture, using aged tap water as a culture medium, was started on May 29, 1967. Twenty-five cladocerans were inoculated into the medium, and by the middle of June their numbers had increased substantially. By July 24, there were great numbers of cladocerans present. This culture was maintained successfully for approximately two and one-half months.

Two attempts to culture cladocerans in Knop's solution (Appendix IV) were tried but little success was achieved. Twenty-five cladocerans were inoculated into one of the cultures on May 29, 1967. By June 2, the organisms had died. Another attempt was made using a different strain of cladocerans on June 8, 1967. These organisms died by June 15.

Greater success was achieved using Brandwein's and Chalkey's solutions (Appendix IV). On June 22, 1967, both cultures were inoculated with twelve cladocerans. By July 4, both cultures appeared to be well established. By July 18, ephippia began to appear on the surface of both cultures. The culture in Chalkey's solution began to die



by July 24, and in Brandwein's solution by July 31. Both cultures remained living for approximately two months.

Several cultures were established using pond water. Cultures or organisms obtained from Grande Prairie, Fort McLeod, Satinwood, Falun, Alix, and Lacombe were established. The cultures were maintained for periods ranging from one and one-half months to four months with two and one-half months being the average length of the period of success. There did not appear to be any correlation between the areas from which the samples were taken; north, south, or central Alberta; and the length of time the culture survived.

One sample containing a small number of crustaceans and filamentous green algae, obtained from a ditch near Lacombe, was established on June 9, 1967. The organisms were cultured in the water in which they were found. No food was added and the jar was kept tightly capped. The organisms survived under these conditions for eight months at which time the cap was removed. The organisms remained living until May 15,, 1968, a total period of over eleven months, at which time the culture was terminated.

#### Sub-Class Malacostraca

It was found that amphipods, such as Gammarus sp., could be cultured without any major difficulties. Several cultures were maintained in 32 oz jars, and one in a two-gallon aquarium. Reproduction often took place in the cultures, resulting in large numbers of the organisms. Aquatic vegetation was added to the culture while a





feeding of hard-boiled egg yolk, added once a week, proved a satisfactory food supplement. The cultures survived for periods ranging from two to eight months, with about five months being the average.

#### Sub-Class Copepoda

It was found that copepods were the easiest crustaceans to culture with very few difficulties being encountered. The most common copepod in the cultures was Cyclops sp., but Osphranticum sp. and Diaptomus sp. were also present in many cultures.

Successful cultures were maintained for as long as eleven and one-half months with about six months being the average. It was found that Cyclops is easier to culture with the method used than either Diaptomus or Osphranticum, although cultures of these were maintained for as long as three months.

Several types of water proved to be useful as a culturing media for copepods. A culture of copepods, obtained from a ditch near Barrhead, was initiated on May 29, 1967, in aged tap water and was maintained successfully for eight months. A culture was maintained in Chalkey's solution for three months, and several were maintained in pond water for eleven months. As with other cultures, distilled water was added as required.

One mixed culture of cladocerans, copepods, and ostracods, collected from Grande Prairie on June 13, 1967, was initiated. The water fouled and the majority of the organisms died by June 15. It





is probable that too many organisms were present in the culture which led to its initial failure. By June 21, the water of the culture began to clear and sweeten. By the middle of July, there were large numbers of copepods present in the culture and the culture was maintained until May 15, 1968, at which time it was terminated. At no time was supplementary food added to the culture. Myriophyllum and filamentous green algae were present in the culture.

A culture containing Cyclops from a ditch near Lacombe was started on June 9, 1967. The culture medium was ditch water and the container was a 32 oz jar. Filamentous algae was placed in the culture jar, and the jar was tightly capped. Copepods were still surviving in the culture after eight months. The cap was then removed and the copepods continued to survive until May 15, 1968, at which time the culture was terminated.

There did not appear to be any correlation with the area of the province from which the samples were taken and the success of the culture.

#### Sub-Class Ostracoda

Several cultures of ostracods were maintained, with two cultures surviving for eleven months. The average length of time successful cultures were maintained was approximately seven months. Only twice did cultures fail to survive for at least three and one-half months. All cultures were in 32 oz jars, but various types of



water were used as culture media. When the addition of water to the cultures was deemed necessary, distilled water was used, except for the aged tap water cultures.

Ten cultures of ostracods contained in jars of pond water were initiated from May 29, 1967, to June 24, 1967. The average life span of the cultures was five months, ranging from one month to eight months. No great difficulties were encountered and it was found that the ostracods are very hardy and easy to raise. Several cultures were maintained using aged tap water as the medium. As with the pond water, no great difficulties were encountered and the cultures were maintained successfully for approximately five months. However, one culture was attempted using Knop's solution as medium, and the organisms died within six days (Appendix IV).

On May 28, 1967, a 4 oz jar of damp mud was taken from the edge of a drying pond. The next day, the mud sample was divided approximately in two, and each half was placed in a 32 oz jar. To one jar, Brandwein's solution was added, and to the other Chalkey's solution. By June 5, a small number of tiny ostracods were present in both cultures. By June 26, pondweed (Potamogeton sp.) had started to grow in Brandwein's solution, while in Chalkey's solution a filamentous algae was present. Both cultures progressed and developed rapidly. More ostracods were produced and those initially present increased in size. From June 5, to the end of September, hard-boiled egg yolk was added to the cultures at least once a week. After that



time, supplementary feeding was stopped. Organisms continued to live in both cultures until the end of January, 1968, at which time the cultures were terminated.

Two cultures were initiated in which there was no supplementary feeding at anytime. One culture was started on June 9, 1967, in pond water with mixed crustaceans and filamentous green algae collected from a ditch near Lacombe. The 32 oz jar was sealed and remained so for eight months. After this time, the jar was opened and ostracods were still present, although they were small in size and number. The jar was left open and the organisms continued to live until May 15, 1968, a total time period of over eleven months.

The second culture was a mixed culture of cladocerans and ostracods collected from Grande Prairie on June 13, 1967. By June 15, the cladocerans had died, probably due to overcrowding, and the water began to foul. By June 21, the water had begun to sweeten and soon after a number of ostracods were present. The organisms continued to survive without any supplementary feeding until the termination of the culture on May 15, 1968. As with the previous culture, the ostracods were quite small in size.

It was found that there was no discernible difference between the length of time a culture survived, and the location from which the sample was taken. Several types of water were used as culture media, and it was found that ostracods appear to survive for longer periods of time in cultures which have a small amount of sediment in





the bottom of the culture dish, regardless of the type of water used.

#### Class Arachnida

Few attempts were made to culture members of the Order Hydracarina. It was found, however, that water mites are quite hardy and can be cultured successfully in pond water for at least six weeks. One culture was started on June 9, 1967, using organisms obtained from Pigeon Lake in central Alberta. The mites were kept in a 32 oz culture jar containing cladocerans which acted as a food supply. The culture remained living until July 21. During July and August of 1968, it was found that mite eggs attached to vegetation hatched out in a culture jar containing pond water. Cultures of this type were kept living for a period of five weeks, using a mixture of crustaceans for a food supply.

#### Class Insecta

It was found that, generally, the most difficult aquatic invertebrates to maintain in the laboratory were the aquatic insect larvae. Some success was achieved, although large numbers of cultures were not attempted. It was found that with some diligence and practice, cultures of some aquatic insects and their larvae can be cultured successfully.

When culturing aquatic insects or their larvae, it is wise to have a good supply of aquatic vegetation in the culture jar.

Myriophyllum, Ranunculus, and Potamogeton seemed to work very well.





It was found inadvisable to place too many organisms in any one culture, and to avoid placing carnivorous species, such as Dytiscus larvae, with herbivores or smaller carnivores. The use of pond water proved quite successful but these organisms appeared to respond negatively to aged tap water or synthetic pond water (Appendix IV). A small amount of sediment in the bottom of the jar appeared favorable for most orders. The cultures were kept at room temperature and in moderate light.

#### Order Coleoptera

On June 23, 1967, a culture of predaceous diving beetle larvae (Dytiscus sp.), was initiated in a 32 oz jar containing pond water and vegetation. In a very short time, the larvae began to cannibalize one another. Within two days, all the larvae in the culture were dead.

During July, 1968, a predaceous diving beetle adult and larva were maintained in the laboratory for a one month period. Both the adult and larva were cultured in one 128 oz jar containing pond water and Myriophyllum. Beetle larvae (Tenebrio sp.) were added every second day to supply the organisms with food (Appendix IV). The larva was seen to feed several times, but the author never witnessed the adult feeding. After approximately one month, both the adult and larva died.

A culture containing several water scavenger beetles (Family Hydrophilidae) collected from a ditch near Peace River on June 13,



1967, was maintained. Vegetation was present in the 32 oz culture jar for food, as members of this family are vegetarians. The culture remained successful for three weeks, after which the beetles began to die.

There are at least thirteen families of aquatic water beetles, some of which are carnivores and some herbivores (Pennak, 1953). When attempting to culture aquatic beetles, or their larvae, it was necessary to first identify the specimens in order to determine their feeding habits. If carnivorous, supplementary feeding of raw liver, mealworms, or smaller aquatic organisms was necessary to achieve any degree of success. If herbivorous, some aquatic vegetation was supplied.

#### Order Collembola

Strictly speaking, the springtails are not truly aquatic, as they never submerge below the surface film of the water. They are more commonly found on the damp shores of aquatic environments, but many can be collected from the surface of the water near shore as well. Collection may be carried out using a simple nylon net, and transferring the organisms to collecting jars.

Three cultures of springtails were maintained during June and July, 1967. Two cultures were initiated in 32 oz jars using aged tap water as a medium. One culture was started on June 23, using organisms collected from a ditch near Milk River. The other culture was



started on June 24 using organisms collected from a slough near Brooks. Springtails remained living in both cultures for approximately one month.

The third culture was maintained in a two-gallon aquarium of lake water with organisms collected from Barnett Lake near Lacombe on May 12, 1967. By the middle of June, the springtails could still be seen jumping from the water's surface and striking the sides of the container. They would then slide down the meniscus of the water and come to rest on the flat surface. When culturing springtails, it was found necessary to maintain a relatively low water level in the container as the organisms can jump several inches from the water's surface.

No supplementary food was added to the cultures. The organisms feed mostly on algae and other plant debris (Pennak, 1953), but an occasional feeding of hard-boiled egg yolk might well prove a satisfactory substitute.

### Order Diptera

Of the Order Diptera, members of the Family Culicidae were cultured. Successful cultures of both mosquitoes (Culex sp.) and phantom midges (Chaoborus sp.) were maintained from the larva through adult stages. A large fingerbowl was found to be a satisfactory container. It must be kept covered at all times to prevent the escape of the adults when they emerge. Cheesecloth or wire





screening works very well. The larvae and pupae of both mosquitoes and phantom midges can be easily collected from the water using a nylon net.

Two cultures of mosquito larvae and pupae were maintained in large fingerbowls. On May 12, six mosquito larvae obtained from a ditch near Lacombe, were culture in a fingerbowl containing some of the ditch water. By May 16, two of the larvae had pupated and emerged as adults, while the remaining four larvae had reached the pupa stage. Four days later, all had emerged as adults.

On May 30, sixteen mosquito pupae were collected from a ditch near Bentley in central Alberta and were placed in a culture of ditch water. By June 2, nine of the organisms, all males, had emerged as adults. By June 5, all of the organisms had emerged as adults, totalling eleven males and five females.

The larvae of the mosquitoes are herbivores and filter out algae and organic debris from the water. It was found that the organisms survived quite well on feedings of hard-boiled egg yolk.

Thre cultures of phantom midges were maintained with varying degrees of success. On May 12, 1967, six larvae, obtained from a ditch near Lacombe, were placed in a two-gallon aquarium containing aquarium water and vegetation. By May 16, four of the larvae had pupated and emerged as adults. The remaining two larvae had died.

On June 13, approximately twelve phantom midge larvae obtained from a pond near Fairview were cultured in a large fingerbowl





containing pond water. On June 27, two of the organisms pupated and emerged as adults. Two more of the larvae had reached the pupa stage. Shortly after that time the culture was accidentally destroyed.

A third culture of larvae obtained from a ditch near Cardston was initiated on June 23, using some of the ditch water as medium. None of the larvae reached the pupa stage, and by July 5, all of the larvae had died.

The larvae of Chaoborus sp. are predatory, feeding on small crustaceans which they catch using their prehensile antennae (Pennak, 1953). When culturing the larvae, a supply of crustaceans such as Daphnia or Cyclops were added as a food supply. In both the Culex and Chaoborus, it was found that the larva stage lasts much longer than the pupa stage. In order to observe complete metamorphosis, it was found necessary to maintain a close watch on the cultures as the pupa stage lasts only a few days.

#### Order Ephemeroptera

Mayfly larvae can be collected using a nylon net and passing it through submerged aquatic vegetation. Mayfly larvae need an abundance of oxygen (Pennak, 1953), therefore it is essential that the number of these organisms be limited in relation to the volume of water in which they are cultured. Most of the larvae are vegetarians, so an adequate supply of aquatic vegetation must be provided.

Three cultures of mayfly larvae were maintained. Two were



initiated on June 9, 1967. One culture was established using two larvae obtained from a ditch near Falun, the second contained one larva obtained from a slough near Satinwood. Both cultures were established in 16 oz jars containing water the organisms were found in plus an abundance of Myriophyllum. On June 28, it was found that one of the larva from Falun had emerged as an adult or subimago. The second larva died two days later. The larva from Satinwood continued to live until early July, a total period of just under one month.

The third culture contained six larvae obtained from a ditch near Fort McLeod on June 23, 1967. The culture was set up in a 32 oz jar containing ditch water and pondweed. The organisms remained active for approximately two weeks, after which they began to die in succession.

#### Order Hemiptera

Three families of water bugs were cultured. A culture containing a backswimmer (Family Notonectidae) obtained from a pond near Rycroft, was established on June 13, 1967, in a 32 oz jar containing pond water and aquatic vegetation. Since backswimmers will float to the surface when they are inactive, it was found advisable to provide a submerged object, such as a stone, which they may rest upon when they are not swimming. One or two small water scavenger beetles were added every four or five days as a food supply. The backswimmer lived



for almost three weeks under these conditions. It seems probable that if a battery jar were used as a container and ample vegetation and food were available, the organisms could be successfully cultured for longer periods of time.

On July 5, several water boatman (Family Corixidae) were obtained from a slough near Nisku and cultured in a 32 oz jar containing slough water. The organisms lived for eighteen days after which time they began to die. Egg yolk was added as a food supply, but the organisms did not appear to feed on it. Boatman feed by scraping decaying material from a muddy substrate into their mouths, using their first tarsi (Pennak, 1953). The culture in which the boatman were contained did not contain a muddy substrate. If the organisms were cultured in a container which had a muddy substrate, it seems probable that the organisms could be kept living for a longer period of time.

During early July, 1968, water striders (Family Gerridae) were kept living for approximately two weeks in a two-gallon aquarium containing pond water. No careful observation of the time factor was maintained on these organisms. However, they were fed on lace wings (Order Neuroptera) which they took readily. The lace wings were killed and dropped on the surface of the water. In a short period of time, a water strider could be seen feeding on the dead organism.

All of the water bugs cultured were collected using a simple nylon dip net and then transferred to culture bottles. It was found





necessary to exercise some care when handling the Notonectidae as they can inflict a nasty "sting" with their mouth parts. Both the Notonectidae and the Corixidae are capable of flight. It was found necessary to keep the cultures covered with cheese cloth or screening.

#### Order Odonata

Little success was achieved in the culturing of damselflies and dragonflies. Several cultures were attempted during June, 1967, in 32 oz jars, but the longest any survived was one week. Vegetation was present in the cultures and pond water was used as a medium, but the organisms usually died in two or three days.

However, during October, 1967, a dragonfly larva was placed in an established five-gallon aquarium containing small crayfish. Mealworms were added weekly as a food supply for the crayfish. Several times it was noted that the dragonfly larva also fed on the mealworms.

The dragonfly larva continued to live in the tank for approximately four months, after which time it disappeared. One can hypothesize that the larva had been captured and eaten by the crayfish, or that it had died and was eaten by the crayfish.

All of the odonate nymphs are carnivorous, and a food supply of other aquatic insects, annelids, or crustaceans must be provided when cultured. As previously mentioned, mealworms appear to provide an adequate food for larger odonate larvae. Collection may be carried out by probing among submerged vegetation using a simple dip net.





### Order Plecoptera

No success was achieved in the culturing of stoneflies. In almost all cases, the larvae were dead, or nearly so, by the time the organisms were brought back to the laboratory. At most, the organisms lived for two or three days. According to Pennak (1953), stonefly larvae need an abundance of oxygen. If the organisms were maintained in an aquarium tank and had a supplementary supply of air added, some success might be achieved. The larvae have various feeding habits, some being carnivorous and some herbivorous. In order to have some success at culturing, the species must be identified in order to decide what type of food must be provided. Collection of the organisms was carried out by handpicking the larvae from the undersides of submerged stones or debris.

### Order Trichoptera

Some success was achieved in the culturing of caddis fly larvae. Five cultures were maintained for varying lengths of time. The first culture was established on June 13, 1967, using specimens obtained from a ditch near Peace River. The organisms were maintained in a 32 oz jar containing some of the ditch water and pondweed. By June 30, one of the larvae had sealed its case and begun to pupate. The remaining larvae died two or three days later.

The second culture was established on June 14, 1967, using specimens obtained from a stream near Valleyview. The organisms



lived in a 32 oz jar until July 4, a period of less than one month.

The third and fourth cultures were started on June 23, 1967, with specimens obtained from Coleman and Pincher Creek. Both groups of organisms were cultured in 32 oz jars containing some of the water in which they were found. The specimens from Coleman died in one week. The specimens from Pincher Creek were collected while they were in the pupa stage, attached to a submerged piece of wood. The piece of wood, with the attached casings, was placed in a culture jar and by July 5, two adults had emerged. By July 21, six more adults had emerged from the casings. No more activity was noticed in the culture for a period of over two months. The culture was then terminated.

The fifth culture was started on July 5 using three larvae obtained from a slough near Nisku. The organisms were placed in a large battery jar containing some of the slough water, mud, and pondweed. The organisms survived until October 3, at which time one larva pupated. Shortly after, the remaining two larvae appeared to die.

Most caddis fly larvae are herbivorous, therefore a good supply of aquatic vegetation must be supplied. The specimens in the battery jar were fed hard-boiled egg yolk which they appeared to utilize. No supplementary food was added to the other caddis fly cultures. Caddis fly larvae were usually collected by handpicking from the substrate or vegetation. It was found that they can also be collected by means of a simple dip net.



## Phylum Vertebrata

### Class Amphibia

Frogs and toads were maintained at all phases in their life cycle, from eggs through to adults. When attempting to hatch amphibian eggs it was found that one must be careful not to place too many eggs in one container. During development, the eggs need a good supply of oxygen, and if too many eggs are present they will die and decompose, fouling the water.

On May 26, eggs of the chorus frog (Pseudacris nigrita) were collected from a pond near Athabasca in northern Alberta. About one dozen eggs were placed in a 32 oz jar containing some water from the pond and pondweed. At this time the animal and vegetal poles of the eggs were clearly visible. Within forty-eight hours, the eggs began to exhibit advanced cleavage stages. On May 29, the tadpoles had hatched and three were placed in each of two 16 oz jars, one containing Chalkey's solution and the other Brandwein's solution. The remaining six tadpoles were placed in a large fingerbowl containing pond water. A small amount of barley pabulum was added to each culture as a food supply for the organisms. On June 9, egg yolk was also added as a supplementary food supply to both 16 oz cultures, but not to the fingerbowl. The tadpoles began to rise to the surface and appeared to be short of oxygen. Therefore, also on June 9, Myriophyllum was placed in each culture.

By June 12, three of the tadpoles in the fingerbowl had died.







The remaining three were transferred to a 32 oz jar of Chalkey's solution and egg yolk was added as a food supplement. The transfer appeared to be beneficial, for within three days the tadpoles became more active and appeared to behave more normally. Egg yolk and pabulum continued to be fed to all three cultures approximately twice a week. When necessary, distilled water was added to maintain a relatively constant water level.

Approximately two months after the cultures were started, most of the tadpoles began to develop hind legs. The organisms did not show much further development. Some of the tadpoles began to die around mid-September. By October 3, there was still one tadpole living in each 16 oz culture, but the tadpoles in the 32 oz culture had all perished. The two remaining tadpoles lived for another two weeks at which time the cultures were accidentally destroyed.

Several species of adult amphibians were kept living in a ten-gallon terrarium containing moss, green plants, soil, rocks, and a pan of water. Chorus frogs, wood frogs (Rana sylvatica), and western toads (Bufo boreas) were found easy to maintain. Leopard frogs (Rana pipiens) did not adapt well to handling and feeding. The first three species mentioned were maintained for about four months without any difficulty and were fed mealworms, earthworms, and raw liver. A feeding of several mealworms per frog, once or twice a week, seemed adequate. For ease of feeding, the animals were often removed from the terrarium two or three at a time, placed on a table top, and live



mealworms placed in front of them. In most cases, the animals began to feed almost immediately. With increased handling, the animals became quite tame and fed readily whenever food was placed in front of them. If live food was not available, the animals would often take a piece of raw liver dangled in front of them.

#### Class Osteichthyes

Many local species of fish were found available and successfully cultured in the laboratory. It was found that overcrowding may prove fatal. About one inch of fish per gallon of water seems a good rule of thumb to establish an aquarium. Aquatic vegetation proved a necessity to provide oxygen and also as a food supply for herbivores. It was noted that if an air-stone is introduced to insure an adequate supply of oxygen the plant Myriophyllum must not be used as the agitation of the water kills plant growth. Also, larger carnivorous species must not be placed in the same tank with smaller carnivores or herbivores.

There are at least five families of fish which will adapt readily to laboratory conditions. Table VI lists the families and genera of fish which can be raised in the laboratory.

Table VI undoubtedly does not include all the fish which could be successfully maintained in the laboratory. It does however, list some which are very common to most of the province of Alberta. Collection can be carried out by dragging a seine or large dip net



through the shallows of lakes or in small streams. The fish are often very wary, and practice may be needed before one becomes proficient at collecting specimens.

TABLE VI

COMMON FISH FOUND IN ALBERTA  
AND CULTURED, 1967-68

FAMILY	GENUS SPECIES	COMMON NAME
Cyprinidae	<u>Semotilus</u> <u>margarita</u>	Northern Pearl Dace
	<u>Notropis</u> <u>hudsonius</u>	Spottail Shiner
	<u>Rhynchithys</u> <u>cataractae</u>	Longnose Dace
Catostomidae	<u>Castostomus</u> <u>castostomus</u>	Northern Sucker
	<u>Castostomus</u> <u>commersonii</u>	Common Sucker
Esocidae	<u>Esox</u> <u>lucius</u>	Northern Pike
Gasterosteidae	<u>Culaea</u> <u>inconstans</u>	Brook Stickleback
	<u>Pungitius</u> <u>pungitius</u>	Nine-spine Stickleback
Percidae	<u>Etheostoma</u> <u>nigrum</u>	Johnny Darter
	<u>Perca</u> <u>flavescens</u>	Yellow Perch

One aquarium containing local species was established on July 13, 1967. Six longnose dace and two common suckers averaging about one and one-half inches in length were collected from Blackmud Creek two miles south of Edmonton. The fish were placed in a five-gallon aquarium containing some of the stream water and aquatic vegetation. An air-stone was added to insure an adequate supply of oxygen. No attempt was made to control the temperature of the tank except that





it was placed in moderate light and did not receive direct sunlight which could raise the water temperature.

The fish were fed baby pablum once or twice a week. Only sufficient food to be consumed in about ten minutes was sprinkled on the surface of the water. Two of the dace and one of the suckers died within one month. The remaining fish appeared healthy. On September 12, 1967, two Johnny darters and two small perch obtained from Lake Wabamun were added to the aquarium. The perch lived about one month before dying. The darters, dace, and sucker remained alive until about the middle of January, 1968.





## CHAPTER VI

### CULTURING LIVE ORGANISMS FROM MUD SAMPLES

#### Method

Thirty-six mud samples were collected from many areas of the province of Alberta to determine whether or not organisms could be cultured using mud and water as a medium. Twenty-four samples were collected from June 9 through to July 5, 1967, inclusive, and twelve from September 7 through to September 9, 1967, inclusive (Table VII).

Each sample was taken from the edge of an aquatic environment where the water had receded, or from the dry bottom of a temporary body of water. The samples were placed in 4 oz collecting bottles and coded as to date and location. The samples were then air-dried by exposing them to the atmosphere in the laboratory at room temperature for at least one month.

On October 13, 1967, each sample was divided approximately into halves. One-half of each sample was capped and placed in a freezing compartment at -15 degrees Centigrade. The other half remained exposed to the atmosphere at room temperature. The samples remained under these conditions for approximately three months.

On January 15, 1968, the samples were removed from the freezer and allowed to thaw out at room temperature. Each sample, both the frozen and dried, were weighed to the nearest tenth of a gram and placed in culture jars, making a total of seventy-two cultures.



TABLE VII

RESULTS OF MUD SAMPLES COLLECTED DURING JUNE, JULY, AND SEPTEMBER, 1967, AND CULTURED IN 1968

No.	DATE OF COLLECTION	LOCATION	WEIGHT IN GRAMS	DRIED OR FROZEN	JAR SIZE IN OZS	VEGETATION CATEGORY	WATER TYPE	DATE OF FIRST SIGHTING, 1968						
								CLADOCERANS	COPEPODS	OSTRACODS	ALGAE	VASCULAR PLANTS	SNAILS	PLANARIANS
1A	June 9	Clive	26.0	D	32	N	AT					Feb.12		
1B	June 9	Clive	22.5	F	16	N	AT					Feb.24		
2A	June 9	Alix	19.2	D	16	S	AT						Feb. 12	
2B	June 9	Alix	15.4	F	32	S	AT					Feb.21	Feb. 1	
3A	June 13	Grande Prairie	26.7	D	32	L	AT					Feb.12		
3B	June 13	Grande Prairie	24.7	F	16	L	AT			Mar. 14		Feb.21		
4A	June 9	Mirror	54.4	D	16	S	AT					Feb.21	Jan. 29	
4B	June 9	Mirror	22.8	F	32	S	AT					Feb.12		
5A	June 13	Beaver Lodge	23.6	D	32	S	AT					Feb.21		
5B	June 13	Beaver Lodge	12.5	F	16	S	AT					Feb.21		
6A	June 9	Falun	22.0	D	16	N	AT					Feb.21		
6B	June 9	Falun	9.8	F	32	N	AT			Apr. 2		Feb.21		
7A	June 13	Rycroft	48.4	D	32	S	AT						Feb. 6	
7B	June 13	Rycroft	21.9	F	16	S	AT							
8A	June 13	Peace River	33.0	D	16	S	AT					Feb.21	Jan. 22	
8B	June 13	Peace River	17.0	F	32	S	AT					Feb.21		
9A	June 13	Valleyview	45.7	D	32	S	AT							
9B	June 13	Valleyview	16.6	F	16	S	AT							
10A	June 13	Fairview	36.7	D	16	S	AT							
10B	June 13	Fairview	20.4	F	32	S	AT					Feb.21	Jan. 24	
11A	June 14	Valleyview	23.2	D	32	L	AT							
11B	June 14	Valleyview	19.1	F	16	L	AT						Jan. 25	
12A	June 18	Brazeau River	65.4	D	16	N	AT					Jan.29		
12B	June 18	Brazeau River	28.4	F	32	N	AT							
13A	June 22	Olds	18.4	D	32	L	AT					Feb.12		
13B	June 22	Olds	11.7	F	16	L	AT	Mar. 14				Feb.21		
14A	June 22	Calgary	30.7	D	16	S	AT							
14B	June 22	Calgary	21.1	F	32	S	AT	Apr. 2		Feb. 1				
15A	June 23	Fort McLeod	42.8	D	32	S	AT	Feb. 6	Apr. 2	Feb. 1				
15B	June 23	Fort McLeod	27.6	F	16	S	AT							
16A	June 23	Fort McLeod	16.2	D	16	L	AT							
16B	June 23	Fort McLeod	12.5	F	32	L	AT							
17A	June 23	Coleman	13.7	D	32	L	AT					Feb.12		
17B	June 23	Coleman	6.3	F	16	L	AT							
18A	June 23	Pincher Creek	33.7	D	16	S	AT							
18B	June 23	Pincher Creek	22.8	F	32	S	AT			Apr. 2		Jan. 24		Feb.10
										Feb. 12				

AT-Aged Tap Water; DI-Distilled Water;  
L-Large Amount of Visible Vegetation Present;

D-Dried Mud Sample;  
N-No Visible Vegetation Present;

F-Frozen Mud Sample;  
S-Small Amount of Visible Vegetation Present



TABLE VII (continued)

No.	DATE DF COLLECTION	LDCATION	WEIGHT IN GRAMS	DRIED OR FROZEN	JAR SIZE IN OZS	VEGETATION CATEGORY	WATER TYPE	DATE DF FIRST SIGHTING, 1968						
								CLADOCERANS	COPEPODS	DSTRACODS	ALGAE	VASCULAR PLANTS	SNAILS	PLANARIANS
19A	June 23	Cardston	30.3	D	32	S	Di			Feb. 1				
19B	June 23	Cardston	16.2	F	16	S	Di			Feb. 1				
20A	June 23	Milk River	55.0	D	16	N	Di				Feb. 21	Feb. 6		
20B	June 23	Milk River	29.7	F	32	N	Di				Feb. 1			
21A	June 23	Taber	28.9	D	32	S	Di			Feb. 6				
21B	June 23	Taber	11.5	F	16	S	Di			Feb. 1				
22A	June 24	Brooks	47.4	D	16	S	Di			Mar. 5				
22B	June 24	Brooks	19.9	F	32	S	Di		Mar. 5					
23A	June 24	Gleichen	26.8	D	32	L	Di		Mar. 14	Feb. 1				
23B	June 24	Gleichen	15.1	F	16	L	Di		Jan. 24	Mar. 5	Feb. 27			
24A	July 5	Nisku	56.9	D	16	S	Di		Jan. 23					Mar. 5
24B	July 5	Nisku	16.3	F	32	S	Di			Feb. 1				
25A	Sept. 7	Hastings Lake	12.7	D	32	L	Di		Feb. 1	Feb. 21	Feb. 1			
25B	Sept. 7	Hastings Lake	6.5	F	16	L	Di			Feb. 1				
26A	Sept. 7	Kinsella	26.1	D	16	L	Di		Jan. 22		Feb. 1			
26B	Sept. 7	Kinsella	14.4	F	32	L	Di		Feb. 2	Feb. 6	Feb. 21			
27A	Sept. 7	Hayter	30.3	D	32	S	Di			Feb. 1				
27B	Sept. 7	Hayter	18.6	F	16	S	Di			Jan. 29				
28A	Sept. 8	Medicine Hat	46.3	D	16	S	Di		Jan. 23	Jan. 29	Feb. 21		Jan. 23	Feb. 10
28B	Sept. 8	Medicine Hat	21.8	F	32	S	Di		Feb. 27	Feb. 1	Feb. 6			
29A	Sept. 8	Calgary	5.8	D	32	L	Di		Jan. 22	Jan. 22	Feb. 6		Feb. 1	
29B	Sept. 8	Calgary	5.4	F	16	L	Di		Feb. 17		Feb. 17			
30A	Sept. 8	Cypress Hills	39.2	D	16	N	Di							
30B	Sept. 8	Cypress Hills	29.3	F	32	N	Di			Feb. 21				
31A	Sept. 8	Taber	30.4	D	32	S	Di		Jan. 23	Jan. 29	Feb. 21	Feb. 21		
31B	Sept. 8	Taber	15.7	F	16	S	Di		Jan. 23		Feb. 21			
32A	Sept. 9	Raymond	23.1	D	16	L	Di					Feb. 6		
32B	Sept. 9	Raymond	10.2	F	32	L	Di				Jan. 29			
33A	Sept. 9	Cardston	45.7	D	32	S	Di		Mar. 14	Feb. 6	Feb. 21			
33B	Sept. 9	Cardston	22.2	F	16	S	Di			Mar. 5				
34A	Sept. 9	Milk River	25.3	D	16	S	Di		Jan. 23	Feb. 6	Jan. 23		Feb. 1	
34B	Sept. 9	Milk River	13.1	F	32	S	Di		Jan. 23	Jan. 29	Jan. 24	Mar. 14		
35A	Sept. 9	Fort McLeod	18.5	D	32	S	Di			Mar. 5	Jan. 23			
35B	Sept. 9	Fort McLeod	7.8	F	16	S	Di			Feb. 13	Jan. 29			
36A	Sept. 9	Calgary	7.3	D	16	L	Di		Jan. 22	Feb. 1	Feb. 1	Feb. 21	Jan. 29	
36B	Sept. 9	Calgary	5.0	F	32	L	Di		Jan. 29	Feb. 21	Feb. 1	Feb. 1	Jan. 29	

AT-Aged Tap Water; Di-Distilled Water;  
L-Large Amount of Visible Vegetation Present;

D-Dried Mud Sample;  
N-No Visible Vegetation Present;

F-Frozen Mud Sample;  
S-Small Amount of Visible Vegetation Present







Both 32 and 16 oz jars were used as containers for the cultures. Sample No. 1 had the dried half in a 32 oz jar and the frozen half in a 16 oz jar. In sample No. 2 the container size was reversed and the dried half was placed in a 16 oz jar, and the frozen half in a 32 oz jar. This practice was followed throughout the treatment of all thirty-six samples.

During the weighing, each sample was coded as to the amount of vegetation present. Three categories were used: no vegetation visible, small amount of vegetation visible, and large amount of vegetation visible. It was fully realized by the investigator that the categories were somewhat subjective, but nevertheless it was felt that such categorization might prove productive of useful information.

Each sample was weighed, coded, and placed in the appropriate culture jar. To the first eighteen samples aged tap water was added while in the remaining eighteen, distilled water was used. No supplementary food was added at any time.

It was found that the cultures required the addition of water from time to time in order to replace that lost by evaporation. Sufficient water was added to restore the water level to its original point at the base of the neck of the jars on February 2, February 22, March 8, April 2, and April 26. The cultures were terminated on May 15, 1968.

The cultures were checked every third day for evidence of macroscopic life. The dates when organisms or plants were first



sighted was noted. Table VII lists the samples and the dates of the first observations of living forms.

## Results and Interpretations

### Overall Success

It was found that plants grew in 51% of the cultures. The most common aquatic vegetation growing in the cultures proved to be filamentous green algae, which were found to be the only vegetation growing in 31% of the cultures. Ten percent of the cultures had an aquatic plant developing, usually Potamogeton sp., or Fontinalis sp., while ten percent of the cultures had both algae and vascular plants present.

Fifty-three percent of the cultures developed animal life, usually crustaceans. Table VIII lists the distribution of crustaceans found in all cultures.

Using Table VIII, it can be seen that the most common crustaceans found were ostracods, which were present in 49% of the cultures. Cladocerans were found in 26% of the cultures and copepods in 20% of the cultures. Besides these, one culture of ostracods also contained a snail, one culture of cladocerans contained a planarian, and one culture containing all three kinds of crustaceans also contained at least three planarians. The planarians were first seen late at night, February 10, after the cultures had been in total darkness for several hours.



TABLE VIII

## DISTRIBUTION OF CRUSTACEANS FOUND IN ALL CULTURES

ORGANISMS	NUMBER OF SUCCESSFUL CULTURES	PERCENTAGE OF TOTAL CULTURES
Cladocerans, Ostracods, and Copepods	13	19
Cladocerans and Ostracods, only	3	4
Cladocerans and Copepods, only	0	0
Copepods and Ostracods, only	1	1
Cladocerans, only	3	4
Copepods, only	0	0
Ostracods, only	18	25
Total	38	53

The organisms which developed in the cultures proved relatively easy to maintain. None of the successful cultures died before May 15, 1968, at which time all the cultures were terminated. It was found, however, that the cultures which contained plants and animals appeared to have a higher concentration of crustaceans present. Thirty-one percent of the cultures developed animal life without the presence of macroscopic plant life, while the remaining 22% of the cultures which developed animal life also developed macroscopic plant life. It appeared that those cultures which contained the largest number of crustaceans also contained water-moss (Fontinalis sp.).





### Comparison of Jar Sizes

Of the seventy-two cultures attempted, thirty-six were in 16 oz jars, and thirty-six in 32 oz jars. It was thought that perhaps the volume of water compared to the surface area might be a factor affecting the success of the culture. The surface area of the 16 oz jar at the base of the neck was 49.9 sq cm, and that of the 32 oz jar, 66.4 sq cm. The volume of the 16 oz jar to the base of the neck was 350 mls, giving a volume to surface area ratio of approximately 7 mls/sq cm. The volume of the 32 oz jar to the base of the neck was 700 mls, giving a volume to surface ratio of approximately 10.5 mls/sq cm. Table IX lists the distribution of crustaceans by jar size.

TABLE IX

#### JAR SIZE AND CULTURE SUCCESS

ORGANISMS	PERCENT SUCCESS		TOTAL
	16 oz jars	32 oz jars	
Cladocerans, copepods, and ostracods	5	14	19
Cladocerans and ostracods, only	4	0	4
Cladocerans and copepods, only	0	0	0
Copepods and ostracods, only	0	1	1
Cladocerans, only	3	1	4
Copepods, only	0	0	0
Ostracods, only	14	11	25
Total	26	27	53





As can be seen from Table IX, little difference was found between the size of the culture jars used and the success of the culture. It must be noted that the volume to surface area ratios did not remain constant. The volume of water continually decreased due to evaporation while the surface area remained the same. The surface area to volume ratio quoted is that of a jar filled to the base of the neck with water. It is the author's opinion that successful cultures of these organisms, under the conditions which prevailed, can be maintained in either 16 or 32 oz jars.

#### Comparison of Dried and Frozen Samples

Approximately half of each sample was frozen and the other half dried. No attempt was made to insure that samples were equal in weight, but all weights were recorded (Table VII). It was assumed that if viable crustacean eggs were present in some numbers originally, each portion of the sample would probably contain some of the eggs. Table X lists the results comparing frozen and dried mud samples.

Again, little difference was noted between the success of dried and frozen cultures. No relationship was noted between the success of the culture and the amount of mud present in the samples treated.



TABLE X

## CULTURING SUCCESS OF FROZEN AND DRIED MUD SAMPLES

ORGANISMS	PERCENT SUCCESS		TOTAL
	Dried	Frozen	
Cladocerans, copepods, and ostracods	10	9	19
Cladocerans and ostracods, only	3	1	4
Cladocerans and copepods, only	0	0	0
Copepods and ostracods, only	1	0	1
Cladocerans, only	1	3	4
Copepods, only	0	0	0
Ostracods, only	10	15	25
Total	25	28	53

Comparison of Success Between Mud Samples Containing  
Different Amounts of Vegetation

When each sample was weighed, a subjective judgment as to the amount of vegetation present was made. If no macroscopic evidence of vegetation was visible, the sample was considered void of vegetation. If vegetation was present, but not conspicuously so, the sample was said to contain a small amount of vegetation. If vegetation was present, and conspicuous, the sample was said to contain a large amount of vegetation. The author realizes the subjectivity of a measurement of this kind, but it was felt that even such categories as these might yield valuable information. Table XI lists the success of cultures with varying amounts of vegetation present.



TABLE XI

COMPARISON OF SUCCESS OF CULTURES CONTAINING MUD  
SAMPLES WITH DIFFERENT AMOUNTS OF VEGETATION

AMOUNT OF VEGETATION	NUMBER OF SAMPLES	NUMBER OF SUCCESSFUL CULTURES	PERCENT SUCCESS
None present, visibly	10	2	20
Small amount	40	24	60
Large amount	22	12	54

It may be concluded that the presence of vegetation in the mud sample increases the chance of the success of the culture.

Comparison of Distilled Water and Aged Tap Water as a Culture Medium

Of the seventy-two cultures established, the first thirty-six were placed in aged tap water as a medium, and the last thirty-six in distilled water. Table XII lists the results of the successful cultures using the two media.

It would appear that a much greater success was achieved using distilled water as a medium rather than aged tap water. Unfortunately, an error was introduced when the cultures were established. Any sample collected before July 5, 1967, was considered to be a spring sample and any collected during September, 1967, was considered to be a fall sample. All of the cultures established in tap water contained mud samples collected in the spring of 1967. Only 33% of the distilled





water samples were spring samples, the remaining 64% were collected during September, 1967. It may well be that the date on which the sample was collected may be a factor partially determining the success of the culture.

TABLE XII

COMPARISON OF SUCCESS OF DISTILLED WATER  
AND AGED TAP WATER AS CULTURE MEDIA

ORGANISMS	PERCENT SUCCESS		TOTAL
	Distilled	Tap	
Cladocerans, copepods, and ostracods	17	2	19
Cladocerans and ostracods, only	3	1	4
Cladocerans and copepods, only	0	0	0
Copepods and ostracods, only	1	0	1
Cladocerans, only	3	1	4
Copepods, only	0	0	0
Ostracods, only	19	6	25
Total	43	10	53

In order to determine whether or not the type of water influences the success of a culture, a control group was established. Twelve cultures were established on February 8, 1968. All samples used portions of the same mud, which had been collected from Calgary on September 9, 1967. This mud sample was selected because of the success already achieved with it. It was known to produce all three types of organisms plus the aquatic plant Fontinalis sp. in a



distilled water culture (Table VII).

Half the samples were air-dried at room temperature for approximately seven months and then frozen for seventy-two hours at -15 degrees Centigrade. The remaining half of the samples were air-dried at room temperature for the same period of time. Four cultures were established in aged tap water, four in fresh tap water, and four in distilled water. The same method of maintaining the control cultures was used as in the original mud cultures. No supplementary food was added. The cultures were placed in moderate light and when necessary, the appropriate water was added to maintain the water levels in the cultures. Table XIII lists the results obtained with the control group. All samples had an original weight of 5 gms.

As can be seen from Table XIII, there was no apparent difference between the success of the culture and the type of water used. Therefore, it seems evident that if similar mud samples are collected and treated under the approximate conditions which prevailed during this experiment, the probability of success would be high. On the basis of the information obtained from the control group, one cannot state that one type of water is better than another as a culturing media; success was achieved using all three water types. All of the cultures contained living organisms until May 15, 1968, at which time the cultures were terminated.



TABLE XIII

RESULTS OF CONTROL MUD SAMPLES COLLECTED FROM CALGARY, SEPTEMBER 9, 1967

NO.	WATER TYPE	JAR SIZE IN OZS	DRIED OR FROZEN	CLADOCERANS	DATE OF FIRST SIGHTING, 1968		VEGETATION
					COPEPODS	OSTRACOIDS	
1	D	16	Frozen	Feb. 12	Feb. 27	Feb. 21	Feb. 19
2	D	32	Frozen	Feb. 17	Feb. 23	Feb. 20	Feb. 21
3	D	16	Dried	Feb. 16	Feb. 21	Feb. 19	Feb. 21
4	D	32	Dried	Feb. 12	Feb. 27	Feb. 21	Feb. 21
5	FT	16	Frozen	Feb. 12	Feb. 21	Feb. 19	Feb. 23
6	FT	32	Frozen	Feb. 13	Feb. 22	Feb. 21	Feb. 21
7	FT	16	Dried	Feb. 16	Feb. 21	Feb. 21	Feb. 22
8	FT	32	Dried	Feb. 16	Mar. 5	Feb. 27	Feb. 21
9	AT	16	Frozen	Feb. 12	Feb. 27	Feb. 27	Feb. 21
10	AT	32	Frozen	Feb. 21	Feb. 13	Feb. 27	Feb. 21
11	AT	16	Dried	Feb. 16	Feb. 27	Feb. 27	Feb. 21
12	AT	32	Dried	Feb. 16	Feb. 23	Feb. 19	Feb. 21

LEGEND:

D - Distilled Water

FT - Fresh Tap Water

AT - Aged Tap Water



### Comparing Success of Fall and Spring Mud Samples

On the basis of information obtained from Tables XII and XIII, it seems reasonable to compare the success of samples collected during the spring and fall of 1967. Any sample which was collected on or before July 5, was considered to be a spring sample. Any sample collected during September, was considered to be a fall sample. Table XIV lists the results obtained using fall and spring samples.

TABLE XIV

#### SUCCESS OF FALL AND SPRING MUD SAMPLES

DATE OF SAMPLE	NUMBER OF SAMPLES	NUMBER OF SUCCESSFUL SAMPLES	PERCENT SUCCESS
Fall	24	21	88
Spring	48	17	35

Table XIV shows that greater success was achieved by using mud samples that were collected during September than with samples collected during the spring. This may partly be explained by the fact that cladocerans and copepods produce two different types of eggs (Pennak, 1953). Cladocerans undergo parthenogenesis during spring and early summer producing a thin-walled "summer egg." During late summer and fall they reproduce sexually and the females develop ephippia, or "winter eggs." Copepods also produce quick developing "summer eggs"





and slower developing, drought resistant, "winter eggs," although parthenogenesis is not common. Most of the copepod winter eggs are laid during late summer and fall. The summer eggs of the cladocerans and copepods cannot stand dessication or freezing, while the winter eggs are capable of withstanding both low temperatures and dry conditions. With this information in mind, greater success might be expected with the culturing of cladocerans and copepods from mud samples collected during the fall months.

Almost all ostracods reproduce by parthenogenesis, and in some species males are unknown (Pennak, 1953). Almost all the eggs produced by ostracods are capable of withstanding drought and freezing temperatures. However, the majority of eggs are laid during late summer and fall. Perhaps the fact that almost all ostracod eggs are capable of withstanding adverse conditions assists in understanding why 88% of the successful spring samples contained ostracods. Sixty-five percent of the successful cultures did not contain any macroscopic animal life except ostracods.

It must be remembered, however, that all of the fall samples were in distilled water, while only 25% of the spring samples fell in this category. However, on the basis of Tables XII and XIII, one can state with some certainty that fall samples tend to be more successful than spring samples.

An attempt was made to culture mud taken from the bottom of a



slough during mid-winter. On February 6, mud samples were taken from the bottom of a slough near Ellerslie in central Alberta. One sample was taken from the middle of the slough which was covered by approximately twenty-four inches of ice. Two other samples were taken from the edge of the slough where the ice was four to six inches deep. The samples were placed in 32 oz jars and ice from the pond was added as a water supply. The cultures were placed in moderate light at room temperature. No supplementary food was added. Weights of the samples were not taken, but the volumes were approximately equal. Table XV lists the results obtained with the cultures.

TABLE XV

RESULTS OF CULTURING MID-WINTER MUD SAMPLES  
COLLECTED FEBRUARY 6, 1968

NO.	THICKNESS OF ICE IN INCHES	DATE OF FIRST SIGHTING, 1968					
		Cladocerans	Copepods	Ostracods	Vegetation	Mayfly Larvae	Mayfly Adults
1	24	Feb. 12	Feb. 21	Feb. 21	Feb. 27	Mar. 5	Mar. 15
2	4-6	Feb. 16	Feb. 21	Feb. 13	Feb. 16		
3	4-6		Feb. 16	Apr. 2		Apr. 2	Apr. 26

As can be seen from Table XV, some success was obtained using mid-winter mud samples. All three cultures contained crustaceans in a



short period of time, although their numbers were small at first. Two cultures developed mayfly larvae which later pupated and emerged as adults. The aquatic plants which developed in two cultures were Potamogeton sp..

### Summary and Conclusions

On the basis of data collected, it can be stated that successful cultures of crustaceans, and occasionally other organisms as well, were achieved using both dried and frozen mud samples. Successful cultures were achieved using both 16 and 32 oz jars. It seems apparent that the presence of vegetation in the mud sample enhanced the chances of success. Success was obtained using both aged tap water and distilled water. On the basis of the results obtained, it is not possible to state which water medium is better, although the control group seems to indicate that any one of the three tested tended to give similar results. It also seems apparent that a greater chance for success is likely when mud samples are collected during the fall, rather than the spring. It was also found that samples collected during mid-winter may be used to initiate successful cultures of crustaceans under the conditions which prevailed during the course of the experiment described earlier.





## CHAPTER VII

### UTILIZATION OF ORGANISMS

Most of the teachers interviewed in the County of Lacombe agreed that the use of local organisms is beneficial in the teaching of biology (Table III). The two main general reasons given for the use of local organisms were that they stimulate student interest and develop in the students an appreciation for the local environment. There are, however, a number of specific ways in which local organisms can be used to help teach biological concepts and relationships. The following chapter attempts to outline some of the ways in which locally obtained specimens can be used.

#### Cell Studies

Protozoans provide excellent specimens for the study of cells and their structure. Many of the protozoans have specialized structures or organelles which students may wish to investigate. To enhance this investigation, many vital stains and dyes are available which may be used in illustrating specific details of cell structure. Vital dyes are particularly useful in that the organism can absorb the stains and still continue their life functions for some time. To prepare the slides, a drop of the stain is placed on each slide and allowed to dry. When the protozoan is ready to be studied, a drop of the culture is added to the slide and the stain will slowly dissolve into it. In many cases a coverslip is not necessary. If one is



desired however, it is advisable to place a small brush bristle between the slide and coverslip to prevent crushing the specimen.

Table XVI lists some of the more common vital stains and the structures which they best illustrate (Brandwein, 1966).

TABLE XVI  
VITAL STAINS AND THE CELL STRUCTURES  
THEY BEST ILLUSTRATE

STAIN	CONCENTRATION	STRUCTURE
Methylene Blue	1:10,000	Nucleus Cytoplasmic Granules Bacteria
Neutral Red	1:3000	Nucleus
Congo Red	1:1000	Food Vacuoles
Janus Green B	1:10,000	Golgi Bodies Bacterial Inclusions
Sudan III	1:50	Neutral Fats
Lugol's Solution	Prepared	Flagella Cilia Glycogen
Nigrosin	Prepared-10%	Cilia
Methyl Green Acetic Acid	Prepared-1%	Nucleus
Acetocarmine	Prepared-45%	Nucleus



Brandwein (1966) also lists several methods by which permanent mounts of stained material can be prepared.

### Functional and Comparative Anatomy

Many organisms can be used to illustrate an organ, or organ-elle, and its relationship to the entire animal. The contractile vacuoles of the Paramecium can be used to illustrate water regulation (BSCS, Yellow Version, 1963; Nuffield, Book IV, 1967). Students can attempt to correlate structures or organisms with their presumed functions (BSCS, Yellow Version, 1963). Many other organisms have structures which are specialized for specific purposes. The tentacles of Hydra or the feet of fairy shrimps can be studied and compared as food gathering structures. Different structures for obtaining oxygen, such as the coxal gills of stonefly larvae, the caudal gills of damselfly larvae, the internal anal gills of dragonfly larvae, and the breathing tube of mosquito larvae can be studied and compared. Specialized structures for feeding, such as the labium of the Odonata or the sickle-shaped mandibles of the Dytiscus larvae can provide contrasting features for study.

The diversity of aquatic invertebrates such as the Hydra, planarian, and Daphnia, provides an excellent opportunity to study comparative anatomy (BSCS, Yellow Version, 1963). Almost any aquatic organism possesses some structures which are unique, providing the opportunity to study functional and comparative anatomy.





## Movement

Many organisms exhibit interesting habits of movement. Paramecium is one which lends itself readily to the study of motion (BSCS, Yellow Version, 1963). In order to study the motion of protozoans, it may be necessary to stain the organisms with a vital dye in order to accentuate the organelles associated with locomotion. This is especially true of the ciliates and flagellates. To observe locomotor details, a drop of a protozoan culture is placed on a slide, covered with a coverslip, and examined under high power. If Paramecium are present, the oblique motion of the cilia will cause the organism to spiral to the left. Wichterman (1953) reports that monovalent cation salts and hydrates, except ammonium sulphate and ammonium acetate, will reverse the ciliary action and cause a rotation to the right. The ciliary action of the oral groove is also worth noting. Carmine powder or India Ink added to the slide will show the water currents set up by the cilia and the students will be able to follow the paths of some of the dye particles. Similar studies may be made with other ciliates and flagellates.

Many of the organisms, especially the ciliated forms, will move too rapidly for beginners to study. Therefore, it may be necessary to slow down their action. To accomplish this, slides can be prepared well in advance of the time of use and much of the water allowed to evaporate. The weight of the coverslip will be enough to impede the movement of many of the organisms. The pressure of the





coverslip flattens the contractile vacuoles enabling them to become more obvious, and their rhythmic pulsations can be seen. If evaporation tends to cause rapid death of the organisms, a drop of methyl cellulose or gelatin may be added, providing a thick medium which will slow down the movements of the organisms (Brandwein, 1966).

To observe amoeba, it is advisable to place a small brush bristle between the slide and coverslip in order to allow freedom of movement for the organism and to witness the action of pseudopodia.

Invertebrate organisms, such as planarians, snails, and leeches, may be observed by watching the underside of the organism as it moves along a piece of glass. A hand lens will usually provide ample magnification for observing this movement.

Hydra are interesting organisms to observe. These invertebrates have two distinctly different types of locomotion; a somersaulting motion and an inch-worm motion. Comparative studies of the two types of movement can be made quite simply.

Fairy shrimps swim with their ventral surface upwards by means of thoracic and abdominal appendages which move in a wave-like fashion. Their long tail-like abdomen is used as a steering oar to enable the organisms to turn quickly. The locomotion of few organisms exhibit the matchless wave-like undulations of these animals. Other crustaceans, such as cladocerans, copepods, and ostracods, have antennae or thoracic appendages adapted for movement of the organism in a variety of ways.



Springtails, whirligig beetles, and water striders have structural features which provide these surface dwellers with interesting and unusual methods of locomotion. These organisms can be observed by means of a hand lens or a low power binocular microscope.

Both damsel and dragonflies belong to the Order Odonata, but their larvae have dissimilar habits of locomotion. The serpentine movement of the damselfly larvae contrasts greatly with both the slow crawling and jet propulsion of the dragonfly larvae.

Water boatman and backswimmers have oar-like legs which are adapted to facilitate locomotion in an aquatic environment. Structural adaptations to a particular environment are illustrated well by these organisms.

The unusual movement of mosquito larvae have given them the common name of wrigglers. The entire body is used in locomotion as these organisms do not have appendages adapted for motion.

Thus it is apparent that almost any aquatic invertebrate can be studied to determine its method of locomotion and the structural adaptations possessed for this purpose. Even relatively sessile organisms, such as Tubifex sp., which will withdraw into mud cases when disturbed, exhibit unusual movements and limited powers of locomotion.

### Tissue Studies

An organism that lends itself readily to tissue studies is



the hydra. These organisms will often contract into a small ball when placed on a slide. In order to relax the hydra they may be placed in a drop of water on a slide resting between two supports. They can be examined through a microscope until the hydra is fully extended and then a lighted match may be placed under the slide for three to six seconds. This will cause the hydra to be relaxed in an extended position. Over or under exposure to the heat will cause contraction or disintegration of the specimen. Trial and error is needed to gain experience in this technique. Draw the excess water towards the tentacles using filter paper or a paper towel. The carbon may be cleaned from the bottom of the slide and the specimen is then ready for tissue examination (Thomas, 1965). If desired, a permanent mount may be made by placing a coverslip over the specimen and sealing in Canada balsam.

To study individual tissues, the living hydra should be fixed and stained. The tissues may be fixed by adding to a slide two drops from a solution of one part glycerin, one part glacial acetic acid, and two parts water. The hydra should be placed in the solution on a slide and allowed to stand for three minutes. If a drop of methyl violet stain is added, allowed to stand for a few minutes and then washed with distilled water, the hydra can then be macerated with dissecting needles. The large epithelio-muscle cells show up very well in this preparation.





## Ingestion

A number of invertebrate organisms may be studied to illustrate the diverse methods by which food can be ingested. Paramecium is one which is often used (BSCS, Yellow Version, 1963). To observe Paramecium feeding, add a small amount of Chlorella or some other unicellular green algae to a concentrated culture of Paramecium on a microscope slide. Paramecium feed by means of ciliary action and the cilia in the oral groove can be seen to create a current of water. The food will move down the oral groove and form food vacuoles at the gullet. In order to show this more clearly, carmine powder or dilute India Ink may be added to the slide. The food balls and food vacuoles will appear a dark color.

Amoeba is an interesting organism in which to study ingestion. The Amoeba can be placed on a slide with a culture of Paramecium or Stentor. A food cup will be formed by the pseudopodia of the Amoeba, and will completely engulf the prey and form a food vacuole. A number of different colors of food vacuoles may be formed caused by the different types of food ingested. It may be possible to see the prey move within the vacuole for a short period of time. Changes can be seen to take place in the food vacuoles over an extended period of time.

Planarians can be observed feeding by placing a number of unfed worms into dishes with some small annelid worms such as Dero or Aulophorus. The action of the proboscis can be seen by using a



binocular microscope or a hand lens. As a substitute for the worms, chopped raw liver may be used as a food source.

The feeding activity of Hydra is very interesting to observe. An unfed Hydra can be placed in a dish with Daphnia or some other small crustacean. The Hydra can be seen using its tentacles to grasp prey. The tentacles will usually be withdrawn and will move the food item towards the hypostome. As the food is ingested, the gastro-vascular cavity becomes distended. A binocular microscope or a hand lens will provide adequate magnification for this purpose.

The action of a snail's radula can be witnessed as the snail crawls along the side of an aquarium. The filtering action of the appendages of fairy shrimps provide interesting opportunities for observation. Other invertebrates, such as the Dytiscus larvae with their sickle-shaped mandibles, and the odonate larvae with their labia, exhibit interesting feeding habits. Any organism which can be induced to feed in the laboratory or classroom can be used in ingestion studies.

### Digestion

Digestion can easily be seen in a variety of protozoans by observing food vacuole changes. Paramecium can be used for this purpose (BSCS, Yellow Version, 1963). To observe food vacuole changes in Paramecium, add milk stained with Congo Red to a slide containing the specimens. Red butterfat globules will be ingested



and form food vacuoles. As acids are secreted into the vacuole to digest the food particles, the indicator will change from red to blue ( $p^H$  of 3). When digestion is complete and acids are no longer being secreted, the vacuoles will return to a reddish color. Similar studies can be made using Amoeba instead of Paramecium.

In small multicellular organisms the path of food through the digestive tract may be observed after ingestion. As with protozoans, indicators make it possible to observe changes that take place in the food as it moves along the digestive tract. For example, if Chorella and Congo Red are added to a culture of Daphnia or rotifers, the digestive tract will slowly change to a red color. After twenty-five or thirty minutes, visible changes can be observed. Areas in the digestive tract which are secreting acids will change from red to blue. Students can note color changes as the food mass moves through the pharynx, stomach, and intestine.

The movement of food along the gut is shown extremely well in the larvae of phantom midges. Because the larvae are relatively transparent, peristalsis can easily be seen. Peristalsis can also be witnessed in cladocerans, but it was found that the phantom midge larvae are better specimens for this purpose.

### Heartbeat and Circulation

Heartbeat and circulation studies can be done with a number of invertebrates. A live clam can be opened by cracking one valve and





cutting the anterior and posterior adductor muscles. Once the mantle has been removed from around the viscera, the pericardial cavity will be exposed. If the heart is bathed in Ringer's solution, it will continue to beat for a period of time. In this example, the contractions of the ventricles are easily seen.

Under microscopic examination the rapidly beating heart of a Daphnia can be easily seen (BSCS, Yellow Version, 1963; BSCS, Green Version, 1963). The specimen can be placed on a slide and covered with a coverslip. A brush bristle placed between the slide and the coverslip will prevent crushing of the specimen. The heart will be found in the dorsal region just posterior to the eye and will probably be beating at the rate of approximately three hundred beats per minute. As an added exercise, the heartbeat can be speeded up by warming the slide or by introducing a trace of 0.01 percent adrenalin to the mount. Students must observe carefully in order to record any changes in heartbeat because of its rapid rate.

Heartbeat can also be observed in snail embryos. An egg mass can be removed from a snail culture and teased apart in a drop of water on a microscope slide. The eggs can be examined under low power without a coverslip.

Aquatic worms are useful in the study of blood circulation. Organisms such as Tubifex have no true heart but have a pair of aortic arches which contract and force blood to flow. The dorsal blood vessel also aids in the pumping action. The blood circulation





is similar in some respects to that of the earthworm, but because of their relative transparency circulation in Tubifex can be seen using a microscope.

The webbing of a frog's foot, or the caudal fin of a fish are excellent for illustrating blood flow in capillaries. The animals are first anesthetized to inhibit movement, and wrapped in moist paper towelling to prevent dessication. With the aid of a low power microscope, the red blood corpuscles can be seen moving through the capillaries.

#### Respiration and Metabolism

One of the easiest ways to illustrate the concept of respiration is to build micro-aquaria using snails as the experimental organism. A pond snail can be sealed in a test tube of water to act as a control specimen. Another snail can be sealed in a test tube of water containing a small portion of a green hydrophyte, such as Elodea. If both samples are placed in sunlight, differences can be noted in a few days. The snail which is the control specimen dies very quickly because of lack of oxygen, while the experimental animal continues to live.

If snails are not available, similar experiments can be set up using protozoans and depression slides. A growing tip from a green hydrophyte can be placed on a depression slide and several drops of a rich culture of protozoans and crustaceans added. Control



groups can be prepared in a similar fashion minus the green plant. The slides can then be kept in moderate light and changes in behavior can be noted using a binocular microscope or a microprojector.

An interesting experiment illustrating varying rates of metabolism can be conducted using planarians. In these organisms, the anterior and posterior portions of the body undergo a faster metabolic rate than the central portion (Nuffield, Book V). The addition of potassium cyanide to the water containing the planarians tends to stop cellular respiration. The anterior and posterior portions of the planarians will disintegrate before the central part. Many interesting questions can be posed as to why this happens.

### Behavior

Planarians can be conditioned to make appropriate responses. A T-shaped maze can be constructed and a piece of raw liver placed in one end of the T while a piece of filter paper soaked in dilute acid or an electric grid is placed at the other end. Planarians placed in the beginning of the maze will "learn" to make the proper response. If it is desirable, conditioning experiments can be combined with studies of regeneration. Once a planarian has learned to make the proper response, it can be cut into two parts. When regeneration is complete, it can be shown that both planarians will now exhibit the correct response (Jacobson, 1962).

An interesting experiment has been conducted using Daphnia. (Nuffield, Book IV, 1967). The experiment is set up to determine the



stimulus that causes Daphnia to congregate near the waters surface under adverse conditions. The results indicate that both light and carbon dioxide concentrations are related to the behavior of the organisms.

Several behavior experiments can be carried out using sticklebacks as experimental animals. Experiments concerning the reactions of sticklebacks to various visible stimuli can be carried out (Nuffield, Book IV, 1967). The mating ritual of sticklebacks is fascinating to observe (Tinbergen, 1952). These animals adapt well to laboratory conditions and are very hardy.

Case construction by caddis fly larvae is an interesting phenomenon to observe. These animals can be stimulated to build unusual cases by removing the original case from the larvae and placing the animals in a jar containing case material which is foreign. It was found that many different kinds of cases will be built under these conditions.

## Tropisms

### Light

Positive phototaxis can be illustrated in the Euglena. A concentrated culture of Euglena may be placed in a fingerbowl and half covered so that light falls on only half the water's surface. If the cover is removed after about ten minutes, a concentration of organisms can be noted on the lighted side of the dish.

Planarians show a negative response to light. These organisms





usually hide beneath rocks or objects which are found in the culture dish. If a culture containing planarians is covered to prevent the entrance of light, the organisms will leave their hiding places and move freely throughout the container. If the cover is removed, the organisms will move away from the light source.

A number of experiments can be tried using Daphnia, or some other crustacean (BSCS, Yellow Version, 1963). By subjecting Daphnia to varying light intensities and wave-lengths, it was found that Daphnia are indeed phototropic and have a greater response to green light than to light of any other wave-length (Personal Communication, 1968).

### Electricity

Brandwein (1966) states that most protozoa will move towards the cathode if they are placed in an electric current. He claims this is due to the electricity having a direct effect on the cilia or other organelles of motion. The cilia on the side of the cathode beat forward; those on the side of the anode beat backward. As a result, the organism moves towards the cathode. A demonstration can be devised to illustrate this phenomenon by partially filling a U-tube with a concentrated culture of Paramecium and connecting a dry cell battery to opposite ends of the U-tube. The Paramecium will tend to cluster around the negative pole. By reversing the current, the Paramecium can be induced to migrate to the opposite end of the U-tube.



### Chemicals

Most protozoans respond to chemical stimuli and exhibit a negative taxis to some weak acids. Paramecium can be shown to collect around some debris or decaying vegetation, probably because of the chemicals diffusing through the water. To illustrate this, a drop of culture fluid containing debris may be mounted on a slide and examined under a microscope. The protozoans will tend to cluster around the food material indicating a positive chemotaxis. A negative chemotaxis can also be illustrated in these organisms by adding a drop of acetic acid to a slide containing a wet mount of Paramecium. The Paramecium can be seen moving away from the diffusing acid. Often the trichocysts will be extruded as the acid concentration becomes stronger (Brandwein, 1966).

Hydra will exhibit a defence mechanism similar to Paramecium when in contact with acid. If a hydra is mounted on a slide, using a brush bristle to prevent crushing of the specimen, and a small amount of acetic acid is added to one side of the coverslip, the hydra will discharge nematocysts. In the case of hydra, the nematocysts are normally used for capturing prey rather than a defense mechanism (Brandwein, 1966).

Planarians also show a negative chemotaxis in the presence of an acid or base. If a small amount of raw liver is placed in a culture of planarians, the animals collect around the food source exhibiting a positive response. If a drop of ammonium hydroxide or



acetic acid is placed on the food source, the planarians will very quickly retreat thereby exhibiting a negative response.

### Touch

Almost all invertebrates show a response when they come into contact with another object. If a slide containing a rich culture of protozoans is touched, the organisms tend to contract immediately. Stentor is a good example to use as it is relatively large and contracts into a small ball. Hydras, planarians, leeches, and all other larger forms of invertebrates will contract or retreat when touched with a dissecting needle.

### Gravity

Paramecium exhibit a negative geotaxis. By using a simple hand lens they can be observed swimming up to the top of a container. If the container is turned upside down, the Paramecium will swim to the opposite ends of the container. Snails, planarians and leeches can also be used to exhibit geotropic responses.

## Reproduction and Life Cycles

### Asexual Reproduction

In a well fed, healthy culture of protozoans, organisms undergoing fission can be found (BSCS, Yellow Version, 1963). By examining a drop of the culture under a microscope, it is possible to find organisms in various stages of asexual reproduction.





Many crustaceans reproduce asexually by means of parthenogenesis. If a Daphnia is placed on a slide and examined under the microscope, the heat from the light will often stimulate the Daphnia to expel parthenogenic eggs from the brood sac.

Asexual reproduction by means of regeneration and budding can be illustrated using Hydra (Nuffield, Book 1, 1967). In a healthy Hydra culture, organisms will often possess buds growing out from the body of the parent. If the buds are removed, or the parent is cut into two parts, regeneration of the missing parts will often take place.

The phenomenon of regeneration is also exhibited by planarians (BSCS, Green Version, 1963). When cut into several parts, these parts will regenerate head ends at varying rates illustrating an anterior-posterior gradient of regenerative powers. Aquatic worms, such as Tubifex, can also be used to illustrate regeneration with cuts through the posterior regions proving most successful (Brandwein, 1966).

#### Sexual Reproduction

Paramecium reproduce sexually by means of conjugation. A good species to illustrate this phenomenon is P. bursaria because of the green color caused by symbiotic algae (Brandwein, 1966). To initiate conjugation, a number of the ciliates may be placed in a culture of dried lettuce leaf powder and distilled water which has been autoclaved. The Paramecium will congregate into large clumps which will





separate into paired conjugation forms in about five or six hours.

Loomis (1959) describes a method by which differentiation of the gonads of hydra can be shown. When the hydra become overcrowded in an aquarium, they will reproduce sexually. Loomis states that sexual reproduction is partially due to increased pressure of carbon dioxide gas dissolved in the water.

It was found that tricladid planarians will reproduce sexually in the laboratory. If a number of planarians are placed in a culture containing hydrophytic vegetation, they will often lay egg cocoons on the vegetation. Some of these will later hatch releasing several young.

Snails, such as Physa or Lymnaea, can be used as a source of living gametes. The multilobed ovatestis, located in the uppermost portion of the spiral of the snail, can be dissected out. If the tissue is mascerated in water on a slide and observed under high power, spheroid eggs and motile sperms may be observed.

Frogs can be stimulated to release eggs and sperms (BSCS, Yellow Version, 1963). Reproductive hormones are obtained by dissecting out pituitary glands from living male and female frogs. The hormones are injected into the same sex of another living frog, and a short time later eggs or sperms will be released. The gametes can be observed under the microscope and can also be used to conduct artificial fertilization experiments.



### Embryology

Both snail and frog eggs can be used to illustrate cell cleavage and embryo development. The early stages of cell cleavage can be observed under a microscope or with a hand lens. Embryo development can be observed through the relatively transparent covering of the eggs until hatching takes place.

Frogs are excellent animals to illustrate metamorphosis (Nuffield, Book I, 1967). By placing tadpoles in a solution containing a small amount of thyroxine, metamorphosis can be speeded up (Nuffield, Book V, 1967). Injections of small amounts of thyroxine into tadpoles will cause structural development to proceed at varying rates.

### Life Cycles

Many aquatic invertebrates can be used to illustrate life cycles. It was found that dipterans were excellent organisms for study. Both mosquitoes and phantom midges were observed to pass through the larva, pupa, and adult stages in the laboratory. Mayfly and caddis fly larvae will also pupate and emerge as adults in the laboratory. However, not all aquatic organisms will exhibit complete life cycles in the laboratory aquarium. Some larvae, such as that of the Dytiscus, must leave their aquatic environment and pupate on land. One must choose carefully which animals to study.



### Population Studies

Gantert (1966) describes an experiment which illustrates the succession of one species over another. Organisms such as protozoans; crustaceans; larvae of mosquitoes, midges, caddis flies, damselflies and dragonflies; nematodes; annelids; and any other aquatic invertebrates one desires to use may be included in the experiment. Different kinds and numbers of organisms are placed in gallon jars with pond plants and then sealed. Records may be kept of the kind and number of each organism placed in the jar. At the end of three or four weeks the ecosystems are examined to determine what organisms are present and their numbers. Many questions can be posed regarding the changes in populations, which may take place.

Mertens (1966) also describes an exercise to illustrate population changes. Several small test tubes of a culture medium containing bacteria, are inoculated with one Paramecium and cultured at twenty-five degrees Centigrade. By examining the test tubes under a binocular dissecting microscope or low power monocular microscope at periodic intervals, such as every twelve hours, counts can be made of the number of protozoans present. Growth curves can then be established for each test tube. Counts may become inaccurate after there has been four or five cell divisions as large numbers of rapidly moving ciliates are difficult to estimate or count.





## Ecology

An overall relationship of organism to environment can be readily illustrated by the use of living aquatic invertebrate forms. A field study of a biotic community, such as a pond, and the organisms found within this community, will help students to see this relationship (BSCS, Green Version, 1963). Organisms can be collected from different areas of the pond, such as on the surface or the bottom, and the data collected can be related to the reasons why organisms prefer particular habitats. For beginners, even the discovery of what organisms can be found in aquatic environments is rewarding in itself (BSCS, Yellow Version, 1963). Nuffield (Book III, 1967) suggests an exercise whereby students discover the distribution of organisms by relating such ecological features as water flow to the presence or absence of specific organisms. Almost any environmental factor could be included in a study of this kind.

Many studies can be conducted to help students understand the problem of water pollution and its effects on aquatic life. Nuffield (Book IV, 1967) suggests using Tubifex, Chironomous larvae, and Eristalis larvae in a study as these organisms are adapted to surviving in oxygen poor habitats. Control and experimental aquaria can be established containing these organisms. Sugar is added to the experimental aquaria as an organic pollutant. Results of the behavior of the organisms in the two aquaria can then be compared and the results graphed or tabulated. Almost any other invertebrate could be



used in similar studies using a wide range of organic and inorganic pollutants.

Classification, and the ways in which organisms can be classified, is enhanced by having students collect organisms from several different phyla and examine them (BSCS, Yellow Version, 1963). While examining organisms, students can learn how to use various keys for identification of specimens, and how to find and use appropriate references which can help to further their investigations.

### Summary

There are a number of incidental advantages of using live organisms. While students are collecting specimens, they will have the opportunity to develop field techniques such as the keeping of careful field notes, accurate observations of organisms and their surrounding environments, and various ways of collecting and transporting specimens. While doing laboratory work, students tend to improve in this ability to use the microscope and to handle and prepare specimens for examination. Many experiments can be attempted by the students which will help to develop their concepts of control and experimental groups and perhaps relate these to the procedures followed by scientists in doing original research.

It must be noted that it is not possible to describe all of the methods and procedures by which aquatic invertebrates may be



utilized to supplement the biology curriculum. Many of the examples cited could be modified, varying conditions such as light, temperature,  $p^H$ , or the addition or deletion of chemicals to obtain new situations and results. A teacher is limited only by his own interest, background, and imagination.



## CHAPTER VIII

### SUMMARY AND CONCLUSIONS

#### Summary

During the 1966-67 school year, biology teachers in the County of Lacombe made very limited use of the local resources available to them. Only a small percentage collected and cultured live organisms. This may be indicative of the degree to which teachers in other areas of the province use local resources in the teaching of biology, but the data gathered does not permit such a generalization.

The most common reasons given for not using local resources were that the collecting of specimens is too time consuming and that many teachers lack the knowledge of what organisms exist in the local environment, where they can be found, and how they can be collected and cultured. The majority of teachers interviewed did, however, express the opinion that the use of locally collected, living specimens are valuable aids in the teaching of biology. The most common reason given by these teachers for using such resources was that it helps to interest, stimulate, and motivate students.

During May and June, 1967, sixty-five aquatic locations in central and southern Alberta were visited to determine what organisms were available for teachers to use. Members of nine different phyla were found to be present in many of the locations sampled. It was found that there were at least twenty-seven different kinds of aquatic





invertebrates common enough to provide easily obtainable specimens for use in the teaching of biology.

During the field investigation, specimens were collected from a variety of localities. It was found that many different kinds of organisms could be obtained from a variety of freshwater environments with only a minimal amount of time and effort required. The equipment needed to collect the specimens was simple and inexpensive.

During the summer of 1967, and the winter of 1967-68, attempts were made to culture collected specimens. It was found that many different kinds of organisms can be maintained in a laboratory for extended periods of time, ranging from a few days to eleven months. It was also found that some organisms, such as fairy shrimps, do not respond well to classroom laboratory conditions.

Mud samples were collected from various locations during May, June, July, and September of 1967. It was found that the mud samples could be used as a source from which live organisms could be obtained during mid-winter months. The freezing and drying of samples, water types, container size, date of collection, and the presence of varying amounts of vegetation in the samples were investigated in an effort to determine their effects upon the success of specific cultures.

It was found that success was achieved with both dried and frozen mud samples. Distilled water and aged tap water provided similar results. The container sizes tested gave little variation in results. Samples collected during the fall appeared more successful



than spring samples, and vegetation appeared to enhance the chances of success of the culture.

A small number of mud samples were collected during February, 1968, to determine whether or not mud collected during mid-winter was capable of providing a source of live specimens. It was found that cultures obtained using mud collected during mid-winter produced living organisms, such as cladocerans, copepods, ostracods and mayflies.

The literature review revealed a variety of methods by which live organisms can be used to aid in the teaching of biological concepts. Much of the material was found to apply to aquatic forms indigenous to Alberta. There seems little doubt that biological concepts can be illustrated and studied directly, using locally collected aquatic forms.

#### Recommendations For Further Study

The study revealed a number of problems for further investigation. A larger and more comprehensive survey of Alberta biology teachers would yield more valid information regarding the use of local resources by biology teachers. The study of other areas and habitats in the province would most certainly yield many forms other than those recorded in this work. Culturing techniques of specimens could be extended and perhaps successful methods of culturing such organisms as fairy shrimps might be discovered.

Culturing of live specimens from mud samples could be expanded.



Carefully controlled experiments to determine what types of water are most successful in culturing organisms could be attempted. Mud samples could be frozen at different temperatures for varying lengths of time to determine what influences these variables have on the success of the cultures. More experimentation is needed using mid-winter mud and ice samples as a source of obtaining live specimens. A quantitative analysis of the organisms present in various types of cultures may yield valuable information regarding population structures. Further careful work needs to be done regarding the relationship of varying amounts of vegetation present in mud samples and the subsequent success of cultures. Experimentation is needed to determine the affects of hydrophytic vegetation on cultures, and to determine which species, if any, tend to enhance the chances of obtaining successful cultures.

The problem of determining additional ways of utilizing living specimens in the classroom could be undertaken. Here, experimental and control classes might be used in an effort to compare the teaching and learning of biological concepts using local resources with the results obtained in using a more traditional approach. Finally, studies similar to the one carried out could be attempted using aquatic vertebrates or terrestrial forms.





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## APPENDIX I

COMMON HYDROPHYTIC PLANTS FOUND  
DURING FIELD INVESTIGATIONS

The following is a list of some common hydrophytic plants used when attempting to culture animal specimens. Only the generic name is given. Brief descriptions of the plants were taken from Cormack (1967), and Engelhardt (1964). For more complete descriptions and illustrations of the plants, the works of these authors should be consulted.

1. Arrowhead (Sagittaria sp.).  
Found in shallow water or slow-flowing muddy water. First leaves are linear; later, long-petioled, sagittate, and towering out of the water. Flower stalk triangular with white flowers in whorls. Recommended for larger aquaria.
2. Bladderwort (Utricularia sp.).  
Found in shallow water in lakes, sloughs, and ditches. A submerged floating hydrophyte possessing finely divided leaves and numerous small bladders one-eighth to three-sixteenth inches long. Not a good plant to use when culturing small crustaceans as the organisms will be trapped by the bladders.
3. Duckweed (Lemna sp.).  
Found floating on quiet water. A small green leaf-like thallus possessing a few very small roots. Individual plants are usually small, but they often form a green carpet over the water's surface. Not a good plant for cultures as very little oxygen is given up to the water when the plant undergoes photosynthesis.
4. Liverwort (Riccia sp.).  
Often found in association with Lemna. Free floating, dichotomously branched pale-green plant. Recommended for most cultures.





5. Pondweed (Potamogeton sp.).  
Several species. Perennial plants with leaves generally submerged and varying from threadlike to broad. Most species will provide a good source of oxygen for cultures.
6. Starwort (Callitriche sp.).  
Found in ditches and ponds. Aquatic or subaquatic plant with floating leaves. Lowest leaves linear; floating leaves set in a rosette. Provides oxygen for cultures of animal organisms.
7. Stonewort (Chara sp.).  
Submerged aquatic algae. Often found in calcareous, eutrophic water. Feels rough and "sandy." Not a good plant for cultures.
8. Water Crowfoot (Ranunculus sp.).  
Found in ponds, ditches, and slow moving streams. Plant often totally submerged except for the flowers. In white water crowfoot the leaves are divided into numerous threadlike filaments. Excellent plants to provide oxygen for aquatic animal cultures.
9. Water Milfoil (Myriophyllum sp.).  
Found in still or slow flowing water. Submerged plants with usually five or six threadlike leaves in whorls along the stem. Excellent plants for cultures, although they will not survive if the water is severely agitated.
10. Water Moss (Fontinalis sp.).  
A bushy, submerged aquatic moss, often found in streams. The plant possesses small acutely-keeled leaves. Excellent plant for aquatic animal cultures.





## APPENDIX II

## INTERVIEW DATA SHEET

No. \_\_\_\_

Date \_\_\_\_ School \_\_\_\_ Grade \_\_\_\_  
Number of classes \_\_\_\_ Size of classes \_\_\_\_

1. What organisms do you collect locally and make use of with your classes in the teaching of biological concepts?

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2. Where and how do you obtain these organisms?

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3. List some organisms which you culture for study. What methods do you use?

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4. How do you use these organisms which you collect locally or culture?

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5. What do you visualize as the major reasons why teachers should use such local resources?

a. 

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b. 

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c. 

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d. 

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e. 

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6. What do you visualize as the major reasons why teachers may not use local resources extensively?

a. 

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b. 

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c. 

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d. 

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e. 

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## APPENDIX III

## LIST OF CULTURING REFERENCES

The following publications contain a variety of methods by which culturing of live organisms may be carried out.

American Association for the Advancement of Science. Culture Methods for Invertebrate Animals. Symposium. Ithaca: Comstock Publishing Company, 1937.

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## APPENDIX IV

## LIST OF CULTURE MEDIA AND FOOD MATERIALS

Culture Media

The following list contains the synthetic pond water solutions which were used in the study.

## Aged Tap Water

Tap water was placed in an open container, preferably an aquarium, and exposed to the atmosphere in the laboratory for at least forty-eight hours before being used.

## Brandwein's Solution (Brandwein, 1966)

Weigh out the following salts, and dissolve them in distilled water to make 1 liter of solution:

NaCl	1.20 g
KCl	0.30 g
CaCl <sub>2</sub>	0.04 g
NaHCO <sub>3</sub>	0.02 g
Phosphate Buffer	50 mls

This is a stock solution. For use, it should be diluted 1:10 with distilled water.

## Chalkey's Solution (Brandwein, 1966).

Combine the following with 1 liter of distilled water.

NaCl	0.1 g
CaCl	0.006 g
KCl	0.004 g





### Knop's Solution (Brandwein, 1966)

Combine the following materials with 1 liter of distilled water.

$\text{KNO}_3$	1 g
$\text{MgSO}_4$	1 g
$\text{K}_2\text{HPO}_4$	1 g

Then add 3 g of  $\text{Ca}(\text{NO}_3)_2$ . This is a six percent stock solution. For immediate use, add 5 l of distilled water to 1 liter of the stock solution. This will yield a 0.1% solution.

### Food Materials

#### Egg-Yolk Paste

To prepare this food supplement, a chicken egg is boiled for several minutes until the yolk is hard. The yolk is separated from the rest of the egg and refrigerated until needed. When a food supplement is required in a culture, a small amount of egg-yolk, about the size of a pea, is mixed with a small amount of water from the culture. When the mixture is the consistency of a thick paste, it may be added to the culture.

#### Mealworm Culture

Larvae of the Tenebrio beetle provide a good food source for larger carnivorous forms, such as frogs and toads. Larvae obtained from a pet shop or supply house can be used to initiate a culture.



The organisms can be placed in a shallow covered pan containing bran, which will act as nutrient for the animals. A paper towel, moistened once a week, and placed on top of the bran will supply enough water for the organisms. The larvae will pupate and emerge as adults. The adults will lay eggs which will hatch out replenishing the larva stage of the life cycle. The culture medium will have to be replaced periodically, about once every two months. Cultures of this type have been maintained for a period of over two years.

#### Pablum

Baby pablum can be used in two ways. Firstly, it can be used to feed herbivorous fish and tadpoles exactly as it comes from the package. Secondly, it can be crushed using a mortar and pestle to form a fine powder which can be used as food by filter feeders, such as clams.











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